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(57) Abstract

The invention provides novel receptors of the tumor necrosis factor receptor family as well as ligands of the tumor necrosis factor family. In addition to the isolated receptors and ligands of the invention, there are provided encoding nucleic acids and related selective binding agents.

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TUMOR NECROSIS FACTOR FAMILY RECEPTORS AND LIGANDS, ENCODING NUCLEIC ACIDS AND RELATED BINDING AGENTS

BACKGROUND OF THE INVENTION

This invention relates to molecules involved in apoptosis and more specifically to tumor necrosis family receptors and ligands.

The tumor necrosis factor (TNF) family of ligands are a superfamily of polypeptides involved in cell-cell signaling. The prototypic TNF molecule was first identified by its ability to kill a variety of transformed cell lines. In addition to their role in cell survival and death, TNF-like molecules and their cognate receptors are now known to be involved in the regulation of a variety of cellular functions including cellular proliferation, differentiation and cytokine secretion.

Ligands of the TNF superfamily are type II membrane glycoproteins with a large C-terminal extracellular domain, a single transmembrane spanning region and a variable cytoplasmic domain. Like the prototypic TNF-α molecule, most are acidic molecules with approximately 20% sequence homology in the extracellular receptor-binding domain. The conservation of the more variable cytoplasmic domains among species homologs indicates that the cytoplasmic domains also can serve an important function in signal transduction. The TNFR ligand family includes TNF-α, TNF-β, LTβ, FAS ligand, CD27 ligand, CD30 ligand, CD40 ligand, OX40 ligand and 4-1BB ligand. Although ligands of the TNF family are typically expressed on the cell surface, soluble forms of TNF-α, TNF-β and FAS ligand also have been identified.

TNF receptor family members generally are type I membrane glycoproteins with sequence homology typically confined to the extracellular domain. This amino-terminal extracellular domain includes a variable number of cysteine-rich pseudo repeats, which are motifs of approximately 40 amino acids containing about six cysteine residues. Numerous cellular and viral members of the TNF receptor superfamily have been described, including TNFR-1 and TNFR-2, TNFR-RP/TNFR-3, nerve growth factor receptor (NGFR), CD27, CD30, CD40, 4-1BB, OX-40, FAS/APO-1 (CD95), DR3 (Wsl-1/APO-3/TRAMP) and DR4. In addition, related viral proteins PV-T2 and PV-A53R encode soluble secreted members of the TNF receptor family.

Several of the tumor necrosis factor receptors function in the process of regulated cell death, termed programmed cell death, or "apoptosis." TNFR-1, FAS/APO-1 and DR3 are TNF receptors that transmit a suicidal signal through a conserved 80 amino acid cytoplasmic region known as a "death domain." Cytoplasmic death-domain containing proteins including FADD/MORT1 (Fas-associated protein with death domain) and TRADD (TNFR-1-associated death domain protein) act as intracellular adaptor molecules in transmitting the ligand-dependent apoptotic signal initiated by a "death receptor." The FAS/APO-1 receptor appears to directly interact with FADD/MORT1, while other death receptors such as TNFR-1 and DR3 associate with TRADD, which acts as an adaptor molecule and recruits FADD/MORT. Despite its sequence homology to the similar domains present in the death receptors, the death domain of FADD does not induce apoptosis when over-expressed in mammalian cells and blocks apoptosis mediated by full-length FADD in a dominant negative fashion. However, FADD possesses another conserved domain denoted a "death effector domain" (DED), which

mediates apoptosis when over-expressed in mammalian cells. Through its DED, FADD binds to homologous domains present in the cysteine protease (caspase) FLICE/MACH1 (caspase 8). FLICE/MACH1 is the most proximal caspase in a cascade of cysteine proteases that are triggered by ligand-dependent activation of the death receptors, ultimating resulting in programmed cell death.

In addition to mediating apoptosis through FADD, the TRADD adaptor molecule can recruit other molecules to the aggregated receptor complex of TNFR-1 or DR3, including the death domain-containing protein RIP (receptor-interacting protein) and TRAF2, which lacks a death domain. While recruitment of FADD leads to activation of caspases and eventual cell death, recruitment of RIP and TRAF2 leads to activation of the NF-κB (nuclear factor kappa-B) pathway, which may protect cells from TNF-induced apoptosis.

Programmed cell death plays a major role throughout development, as well as in the homeostatic control of cell numbers and in defense against intracellular pathogens. Furthermore, abnormalities in programmed cell death contribute to the pathogenesis of a number of diseases. For example, the failure of cells to undergo apoptotic cell death can be involved in cancers such as breast, prostate and ovarian cancer, in autoimmune diseases and in viral infections. Furthermore, a number of diseases are characterized by excessive apoptosis including acquired immunodeficiency syndrome (AIDS); neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and retinitis pigmentosa; osteoporosis; ischemic injury; vasculitis; hepatic necrosis; and cerebral or myocardial infarction. Excessive cell death also can contribute to

organ-specific autoimmune diseases such as Hashimoto thyroiditis. Therapies which are specifically designed to modulate apoptotic pathways can change the natural progression of these and other pathologies characterized by inadequate or excessive apoptosis.

Unfortunately, the ability to provide effective therapeutic intervention is limited by the fact that critical polypeptide receptors and ligands involved in the process of apoptosis remain to be isolated. For example, novel TNF superfamily receptors, which can play a critical role in regulating apoptosis in particular cell types or under certain conditions, remain to be isolated.

Thus, there exists a need to identify and isolate novel TNF superfamily receptors and ligands and their encoding nucleic acids. The present invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

The invention provides novel receptors of the tumor necrosis factor receptor family as well as ligands of the tumor necrosis factor family. In addition to the isolated receptors and ligands of the invention, there are provided encoding nucleic acids and related selective binding agents.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Alignment of the extracellular domains of several representative tumor necrosis family receptors. Alignment was done by pileup program of GCG

software (Genetic Computer Group, Madison, WI). Dominant residues are shown in upper case, or determined by the pretty program of the GCG software.

Figure 2. hAPO8 nucleotide sequence SEQ ID NO:1 and amino acid sequence SEQ ID NO:2.

Figure 3. APO8-related polypeptide (APO8RP) nucleotide sequence SEQ ID NO:3 and amino acid sequence SEQ ID NO:4.

Figure 4. (A) Alignment of the predicted amino acid sequence of APO8 with APO8RP. Identical amino acids are shaded black and homologous residues are shaded gray. Predicted signal peptides, transmembrane regions (TM regions) and death domains are indicated. (B) Sequence alignment of the death domains of APO8, DR4, DR3, TNFR-1 and FAS/APO-1. Residues identical in more than 30% of sequences are shaded black and those homologous in greater than 30% of sequences are shaded gray. Residues corresponding to the site of the *lpr* mutation in the murine FAS receptor are indicated by a Δ symbol.

Figure 5. (A) Induction of apoptosis in MCF-7 cells by overexpression of either the full-length APO8 construct or APO8 mutant constructs. (B) Inhibition of APO8, APO8RP and DR3 induced apoptosis by progressive increasing amounts of dominant negative FADD (DN-FD) in 293T cells. The DNA ratio of receptor to DN-FADD is shown in parenthesis. © Inhibition of APO8-induced apoptosis in MCF-7 cells by dominant negative FADD (DN-FD), dominant negative FLICE (FL-C360S) and the cysteine protease inhibitors CrmA and z-VAD-fmk. (D) APO8 and APO8RP activate the NF-κB pathway. Shown is

the relative luciferase activity from an NF-κB luciferase reporter in 293T cells cotransfected with the indicated reporter construct.

Figure 6. hAPO9 nucleotide sequence SEQ ID NO:5 and amino acid sequence SEQ ID NO:6.

Figure 7. (A) mAPO4-α long nucleotide sequence SEQ ID NO:7 and amino acid sequence SEQ ID NO:8. (B) mAPO4-α short nucleotide sequence SEQ ID NO:9 and amino acid sequence SEQ ID NO:10. © hAPO4-α nucleotide sequence SEQ ID NO:11 and amino acid sequence SEQ ID NO:12. (D) rAPO4-α nucleotide sequence SEQ ID NO:13 and amino acid sequence SEQ ID NO:14. (E) mAP04-γ nucleotide sequence SEQ ID NO:31 and amino acid sequence SEQ ID NO:32.

Figure 8. mAPO4-β nucleotide sequence SEQ ID NO:15 and amino acid sequence SEQ ID NO:16.

Figure 9. hAPO6 nucleotide sequence SEQ ID NO:17 and amino acid sequence SEQ ID NO:18.

Figure 10. Alignment of representative ligands of the tumor necrosis factor family. Residues identical in more than 30% of sequences are shaded black and those homologous in greater than 30% of sequences are shaded gray.

Figure 11. (A) hTNRL1-α nucleotide sequence SEQ ID NO:17 and amino acid sequence SEQ ID NO:20. (B) mTNRL1-α nucleotide sequence SEQ ID NO:21 and amino acid sequence SEQ ID NO:22.

Figure 12. (A) hTNRL1-β nucleotide sequence SEQ ID NO:23 and amino acid sequence SEQ ID NO:24. (B) mTNRL1-β nucleotide sequence SEQ ID NO:25 and amino acid sequence SEQ ID NO:26.

Figure 13. (A) hTNRL3 nucleotide sequence SEQ ID NO:27 and amino acid sequence SEQ ID NO:28. (B) mTNRL3 nucleotide sequence SEQ ID NO:29 and amino acid sequence SEQ ID NO:30.

Figure 14. (A) Induction of apoptosis in 293T cells by expression of full-length human APO4 (hAPO4). Inhibition of APO4-induced apoptosis by various inhibitors of the caspase or JNK pathway are also shown. (B) hAPO4 activates the JNK pathway. Shown is the relative luciferase activity from a c-Jun transactivating reporter in 293T cells cotransfected with hAPO4 in the presence or absence of the indicated inhibitor plasmid (JBD of JIP-1, MEKK1-D1369A, or DN-TRAF2). (C) hAPO4 activates the NF-κB pathway. Shown is the relative luciferase activity from an NF-κB reporter construct in 293T cells cotransfected with hAPO4 and empty vector, dominant negative TRAF2 (DN-TRAF2) or IκBα-S32/36A.

DETAILED DESCRIPTION OF THE INVENTION

Members of the TNFR family are type I transmembrane receptors characterized by the presence of cysteine-rich pseudo-repeats in the extracellular domain. These cysteine rich regions, which are motifs of approximately 40 amino acids with about 6 cysteines, are involved in ligand binding. The average homology in the cysteine-rich extracellular region is in the range of 25% to 30%. In addition to a signal peptide, a large extracellular domain and hydrophobic transmembrane

region, receptors of the TNF family have cytoplasmic domains, which function in transmitting a ligand-dependent signal.

The TNF receptor superfamily contains at least 10 different cellular membrane proteins and several viral proteins encoding TNF receptor related molecules. The first cloned receptor of this family was the nerve growth factor receptor (NGFR), and the TNF receptor superfamily of type I transmembrane receptors now includes TNFR-1 and TNFR-2, TNFR-RP/TNFR-3, nerve growth factor receptor (NGFR), CD27, CD30, CD40, 4-1BB, OX-40, FAS/APO-1 (CD95), DR3 (Wsl-1/APO-3/TRAMP) and DR4. In addition, related viral proteins PV-T2 and PV-A53R encode soluble secreted members of the TNF receptor family. Several members such as TNFR-1, TNFR-2 and NGFR have a broad tissue distribution, while CD27, CD30, CD40, 4-1BB and OX40 are chiefly restricted to cells of the lymphoid or hematopoietic systems.

Table 1				
PERCENTAGE AMINO ACID IDENTITY AND SIMILARITY BETWEEN VARIOUS RECEPTORS IN THE EXTRACELLULAR DOMAIN				
Receptor	hAPO8 (SIN:2)	hAPO9 (SIN:6)	mAPO4-αL (SIN:8)	hAPO6 (SIN:18)
TNFR1	27.3 (46.5)	21.8 (40.0)	20.1 (39.0)	17.0 (32.0)
TNFR2	25.8 (45.1)	21.9 (41.0)	22.4 (41.4)	33.1 (45.0)
Fas	25.1 (45.5)	27.2 (40.9)	23.0 (43.4)	20.4 (38.7)
DR3	21.5 (40.2)	21.6 (36.6)	23.6 (45.9)	28.4 (49.6)
DR4	58.1 (70.6)	57.0 (71.7)	20.4 (36.6)	16.8 (33.7)
* Percentage similarity is shown in parenthesis				

The present invention is directed to the discovery of several new receptors belonging to the TNF family, designated an APO8 polypeptide, an APO8 related polypeptide (APO8RP), an APO9 polypeptide, an APO4 polypeptide, and an APO6 polypeptide. In general, the receptors of the invention are transmembrane proteins with large extracellular ligand-binding domains sharing 16-60% sequence identity in this region with other members of the TNF receptor family (Figure 1 and Table 1 above). A preferred method for determining amino acid identity is by pairwise alignment using the gap program of GCG software (Genetic Computer Group, Madison, WI) using the default parameters.

The present invention provides an isolated APO8 polypeptide, which is a novel TNF receptor having substantially the same amino acid sequence as APO8, or an active fragment thereof. An isolated APO8 polypeptide of the invention can have substantially the same amino acid sequence as the APO8 sequence SEQ ID NO:2 shown in Figure 2.

An exemplary human APO8 polypeptide (hAPO8) is provided herein. hAPO8 is a widely expressed polypeptide containing structural features reminiscent of known "death receptors," including an intracellular death domain. As disclosed herein, overexpression of hAPO8 results in apoptosis, which is caspase-dependent and also dependent upon the FADD/MORT adaptor molecule (Example II). hAPO8 also activates NF-κB expression through the TRADD adaptor molecule and, thus, can have anti-apoptotic activity. As further disclosed herein, hAPO8 interacts directly with TRADD, thereby recruiting FADD/MORT. The hAPO8 polypeptide of the invention can be useful in identifying selective enhancers or inhibitors of APO8 activity, which can be used as novel therapeutics for the management of APO8-mediated diseases or diseases involving the dysfunction of programmed cell death. In addition, nucleic acid molecules encoding hAPO8 or apoptotic fragments of hAPO8 can themselves be used as inducers or inhibitors of programmed cell death.

The term "isolated," as used herein in reference to a polypeptide of the invention, means a polypeptide that is in a form that is relatively free from contaminating lipids, unrelated polypeptides, nucleic acids and other cellular material normally associated with the polypeptide in a cell.

As used herein, the term "APO8" means an APO8 polypeptide and includes polypeptides having substantially the same amino acid sequence as the hAPO8 polypeptide having amino acid sequence SEQ ID NO:2. hAPO8 is a transmembrane protein of 411 amino acids having the amino acid sequence shown in Figure 2. hAPO8 contains an N-terminal signal peptide of 51 amino acids, a large extracellular domain with characteristic TNF receptor family cysteine-rich regions, a hydrophobic transmembrane domain of 27 amino acids (residues 185 to 212) and a C-terminal cytoplasmic domain (residues 213 to 411). The APO8 cytoplasmic domain includes a "death domain," which is a conserved domain of about 80 amino acids present in pro-apoptotic TNF receptors such as TNFR-1, FAS/APO-1 and DR3 and that is required for apoptosis. Death domains also are present in several cytoplasmic proteins, and a homotypic association between death domains appears responsible for the interaction of these cytoplasmic proteins with TNFR-1, FAS/APO-1 and DR3.

As disclosed in Example I, hAPO8 RNA is expressed in a variety of tissues, with a particularly high level of expression observed in peripheral blood lymphocytes (PBL). The results disclosed in Example II demonstrate that the hAPO8 polypeptide of the invention exhibits caspase-dependent apoptotic activity and activates NF-κB gene expression. Thus, hAPO8 is a TNF family receptor, which, like TNFR-1 and DR3, activates NF-κB expression and promotes apoptosis through a cascade of cysteine proteases.

The term APO8 encompasses a polypeptide having the sequence of the naturally occurring human APO8 polypeptide (SEQ ID NO:2) and is intended to include

related polypeptides having substantial amino acid sequence similarity to hAPO8 (SEQ ID NO:2). Such related polypeptides exhibit greater sequence similarity to hAPO8 than to other polypeptides containing cysteine-rich pseudo-repeats or other death domain containing polypeptides and include alternatively spliced forms of hAPO8, species homologues, and isotype variants of the amino acid sequence shown in Figure 2. The hAPO8 polypeptide disclosed herein has about 58% amino acid identity and about 71% amino acid similarity with the Death Receptor (DR4). As used herein, the term APO8 describes polypeptides generally having an amino acid sequence with greater than about 65% amino acid sequence identity with hAPO8 (SEQ ID NO:2), preferably greater than about 75% amino acid identity with hAPO8 (SEQ ID NO:2), more preferably greater than about 85% amino acid identity with hAPO8 (SEQ ID NO:2), and includes polypeptides having greater than about 90%, 95% or 97% amino acid identity with hAPO8 (SEQ ID NO:2).

The present invention also provides active fragments of the APO8 polypeptide of the invention. In general, the term "active fragment" means a polypeptide fragment having substantially the same amino acid sequence as a portion of the indicated polypeptide of the invention, provided that the fragment retains at least one biological activity of the polypeptide. A portion of a polypeptide generally has an amino acid sequence of about 15 to about 400 contiguous residues and can have, for example, an amino acid sequence of at least about 18, 20, 25, 30, 35, 40, 50, 100, 150 or 200 contiguous residues. As described further below, the invention also provides active segments of the APO8 polypeptide of the invention. In general, an active segment is characterized, in part, by having substantially the same

amino acid sequence as a portion of the indicated polypeptide. Such an active segment generally has an amino acid sequence of about 15 to about 400 contiguous residues and can have, for example, an amino acid sequence of at least about 18, 20, 25, 30, 35, 40, 50 100, 150 or 200 contiguous residues.

As used herein, an "active fragment of an APO8 polypeptide" is synonymous with "active fragment of APO8" or "active APO8 fragment" and means a polypeptide fragment having substantially the same amino acid sequence as a portion of an APO8 polypeptide, provided that the fragment retains at least one biological activity of an APO8 polypeptide. An active fragment of an APO8 polypeptide can have, for example, an amino acid sequence that is identical or substantially the same as a portion of the amino acid sequence of hAPO8 (SEQ ID NO:2), provided that the fragment retains at least one biological activity of an APO8 polypeptide. A biological activity of APO8 can be, for example, the ability to bind ligand, bind TRADD or RIP, induce or suppress apoptosis, activate NF-κB expression, or induce or suppress cell proliferation, differentiation or cytokine secretion. Particularly useful active fragment of APO8 are polypeptide fragments having pro-apoptotic or anti-apoptotic activity. A biological activity of an APO8 polypeptide or fragment can be routinely assayed; for example, apoptotic activity can be analyzed by transfecting an APO8 encoding nucleic acid and measuring the number of cells with apoptotic morphology, as set forth in Example II.

An "active fragment of an APO8 polypeptide" also can be an active APO8 segment, which is a polypeptide portion having substantially the same amino

acid sequence as a portion of an APO8 polypeptide, provided that the segment does not consist of the identical amino acid sequence encoded by an expressed sequence tag having GenBank accession number AA223122 or AA232440 and provided that the segment retains at least one biological activity of an APO8 polypeptide. An active APO8 segment can have, for example, an amino acid sequence that is identical or substantially the same as a portion of the amino acid sequence of hAPO8 (SEQ ID NO:2), provided that the segment does not consist of the identical amino acid sequence encoded by an expressed sequence tag having GenBank accession number AA223122 or AA232440 and provided that the segment retains at least one biological activity of an APO8 polypeptide.

In one embodiment, the invention provides active fragments that are soluble, extracellularly expressed forms of the receptors of the invention. Such a soluble active fragment includes the receptor ligand binding domain. Such a soluble active fragment can be an inhibitory polypeptide that binds ligand and opposes the biological function of full-length or membrane-bound receptor. For example, as disclosed herein, APO8 induces caspase-dependent apoptosis when expressed in BHK cells; a soluble form of the APO8 receptor can compete for binding to an APO8 ligand and reduce or inhibit the pro-apoptotic activity of APO8. Another use of such soluble forms of the receptors of the invention can be to transduce a signal across the surface of a cell which expresses the cognate ligand of the receptor. One skilled in the art understands that a soluble receptor can act in a systemic fashion, for example, to block the activity of full-length or membrane-bound receptors wherever the full-length receptor is expressed.

As used herein, the term "soluble" refers to a polypeptide that is not membrane bound. A particularly useful soluble polypeptide of the invention is secreted and, thus, expressed extracellularly.

Provided herein is a soluble APO8 active fragment that includes an APO8 ligand binding domain. A soluble APO8 active fragment of the invention can be, for example, a truncated polypeptide encoding the extracellular domain of APO8. Such a soluble APO8 active fragment can have, for example, substantially the same amino acid sequence as the signal peptide and ligand-binding domain of hAPO8 (amino acids 1 to 184 of hAPO8 shown in Figure 1). One skilled in the art understands that a soluble APO8 active fragment is distinguished from a membrane-bound fragment by the deletion or inactivation of the transmembrane domain, which is shown as amino acids 185 to 212 in Figure 2. A soluble APO8 active fragment also can be, for example, an active APO8 segment. Active APO8 segments are described hereinabove.

The present invention also provides an isolated APO8-related polypeptide, which is a novel TNF superfamily receptor having substantially the same amino acid sequence as APO8 or an APO8-related polypeptide. An isolated APO8 related polypeptide of the invention can have substantially the same amino acid sequence as the APO8 sequence SEQ ID NO:2 shown in Figure 2 or substantially the same amino acid sequence as the APO8RP sequence SEQ ID NO:4 shown in Figure 3. An APO8-related polypeptide is characterized, in part, by the ability to induce FADD-dependent apoptosis or the ability to activate NF-κB. As disclosed herein, APO8 and APO8RP activate NF-κB by a TRADD dependent pathway. As further

disclosed herein, APO8 and APO8RP interact directly with TRADD in co-immunoprecipitation experiments, and FADD is indirectly recruited to APO8 or APO8RP through TRADD. An APO8-related polypeptide of the invention can be useful in diagnosing an APO8-related polypeptide-mediated disease or in treating or reducing the severity of such a disease, as described further below.

hAPO8 related polypeptides include hAPO8RP, a polypeptide of 410 amino acids, and hAPO8, a polypeptide of 411 amino acids. hAPO8RP (SEQ ID NO:4) and hAPO8 (SEQ ID NO:2) share an amino-terminal signal peptide of 51 amino acids with about 53% amino acid identity (see Figure 4A). An APO8 related polypeptide of the invention is characterized, in part, in that the amino-terminal 50 residues of the mature polypeptide (residues 52 to 101) have at least about 30% amino acid identity with SEQ ID NO:2. Although the DR4 sequence described in Pan et al., Science 276:111-113 (1997) shares some similarity with hAPO8 and hAPO8RP, the DR4 sequence has only about 28% amino acid identity in the amino-terminal region of the mature polypeptide (shown as SEQ ID NO:2). Thus, DR4 is not an APO8-related polypeptide, as defined herein.

The term APO8-related polypeptide encompasses a polypeptide having the sequence of the naturally occurring human APO8 polypeptide (SEQ ID NO:2) or the naturally occurring APO8-related polypeptide (APO8RP; SEQ ID NO:4) and is intended to include related polypeptides having substantial amino acid sequence similarity to hAPO8 (SEQ ID NO:2) or hAPO8RP (SEQ ID NO:4). Such related polypeptides exhibit greater sequence similarity to hAPO8 or hAPO8RP than to other polypeptide containing cysteine-rich pseudo-repeats such as TNFR-1 or DR4 and include alternatively spliced forms of hAPO8 and hAPO8RP,

species homologues, and isotype variants of the amino acid sequences shown in Figures 2 and 3A. As used herein, the term APO8-related polypeptide describes a polypeptide generally having an amino acid sequence with greater than about 50% amino acid sequence identity with hAPO8 (SEQ ID NO:2) and, in particular, having a sequence in which the amino terminal 50 residues of the mature polypeptide have at least about 30% identity with amino acids 52 to 101 of SEQ ID NO:2. An APO8-related polypeptide preferably has an amino acid sequence in which the amino terminal 50 residues of the mature polypeptide have at least about 50% amino acid identity with amino acids 52 to 101 of SEQ ID NO:2, more preferably greater than about 75% amino acid identity with amino acids 52 to 101 of SEQ ID NO:2, and can be a polypeptide in which the amino terminal 50 residues of the mature polypeptide have greater than about 85%, 90%, 95% or 97% amino acid identity with amino acids 52 to 101 of SEQ ID NO:2. One skilled in the art understands that the term APO8-related polypeptide encompasses fusion proteins, in which a heterologous polypeptide sequence is fused N- or C- terminally, or is fused internally, for example, near the cleavage site of the signal peptide.

The present invention also provides an isolated APO9 polypeptide having substantially the same amino acid sequence as APO9, or an active fragment thereof. An isolated APO9 polypeptide of the invention can have substantially the same amino acid sequence as the human APO9 sequence SEQ ID NO:6 shown in Figure 6.

As used herein, the term "APO9" means an APO9 polypeptide and includes polypeptides having substantially the same amino acid sequence as the hAPO9 polypeptide having amino acid sequence SEQ ID NO:6. A

short, alternatively spliced form of hAPO9 having 259 amino acids is shown in Figure 6. This hAPO9 sequence contains an amino-terminal signal peptide (residues 1 to 22), a large extracellular ligand binding domain with characteristic TNF receptor family cysteine-rich regions (residues 23 to 241) and a hydrophobic membrane-anchoring domain (residues 242 to 259) at the 3' end. The hAPO9 extracellular domain has significant homology to other TNF receptor polypeptides as shown in Figure 1. Human APO9 is most closely related to the DR4 death receptor, with 57% amino acid sequence identity and 71% amino acid sequence similarity in the extracellular domain. A stop codon is present immediately after the membrane-anchoring domain such that the short form of hAPO9 shown in Figure 6 lacks a cytoplasmic domain. Alternatively spliced, full-length forms of APO9 having a cytoplasmic signaling domain can mediate apoptosis; their function can be opposed by the short APO9 form shown in Figure 6.

The term APO9 encompasses a polypeptide having the sequence of the naturally occurring human APO9 polypeptide (SEQ ID NO:6) and is intended to include related polypeptides having substantial amino acid sequence similarity to hAPO9 (SEQ ID NO:6). Such related polypeptides exhibit greater sequence similarity to hAPO9 than to members of the TNF receptor superfamily and include alternatively spliced forms of hAPO9, full-length forms having a cytoplasmic signaling domain, species homologues, and isotype variants of the amino acid sequence shown in Figure 6. Such full-length forms or APO9 species homologues can be readily obtained by the skilled artisan using routine molecular techniques, for example, by screening an appropriate cDNA library with a portion of SEQ ID NO:5 as a probe. As used herein, the term APO9 describes polypeptides generally having an

amino acid sequence with greater than about 65% amino acid sequence identity in the extracellular domain and transmembrane domain with hAPO9 (SEQ ID NO:6), preferably greater than about 75% amino acid identity in the extracellular domain and transmembrane domain with hAPO9 (SEQ ID NO:6), more preferably greater than about 85% amino acid identity in the extracellular domain and transmembrane domain with hAPO9 (SEQ ID NO:6), and includes polypeptides having greater than about 90%, 95% or 97% amino acid identity in the extracellular domain and transmembrane domain with hAPO9 (SEQ ID NO:6).

The present invention also provides active fragments of the APO9 polypeptide of the invention. As used herein, the term "active fragment of an APO9 polypeptide" is synonymous with "active fragment of APO9" or "active APO9 fragment" and means a polypeptide fragment having substantially the same amino acid sequence as a portion of an APO9 polypeptide, provided that the fragment retains at least one biological activity of an APO9 polypeptide. An active fragment of an APO9 polypeptide can have, for example, an amino acid sequence that is identical or substantially the same as a portion of the amino acid sequence of hAPO9 (SEQ ID NO:6), provided that the fragment retains at least one biological activity of an APO9 polypeptide. A biological activity of APO9 can be, for example, the ability to bind ligand, the ability to induce or suppress apoptosis or the ability to induce or suppress cell proliferation, differentiation or cytokine secretion. A biological activity of an APO9 polypeptide or fragment can be routinely assayed; for example, apoptotic activity can be analyzed by transfecting an APO9 encoding nucleic acid and measuring the number of cells with apoptotic morphology, as set forth in Example II.

An "active fragment of an APO9 polypeptide" can be an active APO9 segment, which is a polypeptide portion having substantially the same amino acid sequence as a portion of an APO9 polypeptide, provided that the segment does not consist of the identical amino acid sequence by an expressed sequence tag having GenBank accession number AA031883, AA150849, T71406 or R10995 and provided that the segment retains at least one biological activity of an APO9 polypeptide. An active APO9 segment can have, for example, an amino acid sequence that is identical or substantially the same as a portion of the amino acid sequence of hAPO9 (SEQ ID NO:6), provided that the segment does not consist of the identical amino acid sequence encoded by an expressed sequence tag having GenBank accession number AA031883, AA150849, T71406 or R10995 and provided that the segment retains at least one biological activity of an APO9 polypeptide.

In one embodiment, the invention provides a soluble APO9 active fragment that includes an APO9 ligand binding domain. A soluble APO9 active fragment of the invention can be, for example, a truncated polypeptide encoding the extracellular domain of APO9. An exemplary soluble APO9 active fragment is provided in Figure 1 as amino acids 1 to 241 of hAPO9 and includes the signal peptide and ligand binding domain. One skilled in the art understands that a soluble APO9 active fragment is distinguished from a membrane-bound fragment of APO9 by the deletion or inactivation of the hydrophobic membrane-anchoring domain, shown as amino acids 242 to 259 in Figure 6. A soluble APO9 active fragment also can be, for example, an active APO9 segment. Active APO9 segments are described hereinabove.

The present invention also provides an isolated APO4 polypeptide having substantially the same amino acid sequence as an APO4 polypeptide, or an active fragment thereof. An isolated APO4 polypeptide of the invention can have substantially the same amino acid sequence as murine APO4-αL (SEQ ID NO:8) shown in Figure 7A, murine APO4-αS (SEQ ID NO:10) shown in Figure 7B, human APO4-α (SEQ ID NO:12) shown in Figure 7C, or rat APO4-α (SEQ ID NO:14) shown in Figure 7D. An isolated APO4 polypeptide of the invention also can have substantially the same amino acid sequence as murine APO4-β (SEQ ID NO:16) as shown in Figure 8.

As used herein, the term "APO4 polypeptide" means an APO4-α or APO4-β polypeptide and includes polypeptides having substantially the same amino acid sequence as the mAPO4-αL polypeptide (SEQ ID NO:8), the mAPO4-αS polypeptide (SEQ ID NO:10), the hAPO4-α polypeptide (SEQ ID NO:12), the rAPO4-α polypeptide (SEQ ID NO:14) or the mAPO4-β polypeptide (SEQ ID NO:16). Murine APO4-α is an alternatively spliced protein including an amino-terminal signal peptide, cysteine-rich pseudo-repeats characteristic of the TNF receptor family and a highly hydrophobic stretch of amino acids representing a transmembrane domain. The short mAPO4-α isoform (mAPO4-αS) is encoded by an open reading frame of 214 amino acids and includes a short cytoplasmic tail, while the long mAPO4-αL isoform (mAPO4-αL) is a polypeptide of 416 amino acids with a unique cytoplasmic tail having no significant homology to other members of the TNF receptor family. The truncated mAPO4-αS polypeptide can compete for binding to an APO4-α ligand, thereby opposing the function of a long form of APO4-α such as mAPO4-αL.

Human APO4-α (hAPO4-α) is another APO4 polypeptide of the invention. The nucleotide sequence encoding hAPO4-α is shown in Figure 7C. This sequence encodes an hAPO4-α polypeptide of 423 amino acids with 70.7% amino acid identity and 81.1% amino acid similarity to mAPO4-α. As shown in Figure 7C, the hAPO4-α polypeptide possesses a signal peptide (residues 1 to 23), a putative transmembrane domain (residues 178 to 191) and the characteristic cytoplasmic domain of APO4 polypeptides. Rat APO4-α (rAPO4-α) is another APO4 polypeptide of the invention. The available sequence of the rAPO4-α cDNA is shown in Figure 7D. The cytoplasmic tail of rAPO4-α contains the unique APO4 cytoplasmic domain and is 80% identical with mAPO4-α at the amino acid level. Murine APO4-β (mAPO4-β) is another APO4 polypeptide of the invention; the available sequence of mAPO4-β is shown in Figure 8. Finally, murine APO4-γ (mAPO4-γ) is another APO4 peptide of the invention whose sequence is shown in Figure 7E. mAPO4-γ lacks a transmembrane domain and therefore represents a soluble receptor which can compete for binding to the APO4 ligand, thereby opposing the function of the long forms of mAPO4 such as mAPO4-αL.

The term APO4 polypeptide encompasses a polypeptide having the sequence of naturally occurring murine APO4-αL (SEQ ID NO:8) or APO4-αS (SEQ ID NO:10), naturally occurring human APO4-α (SEQ ID NO:12) or rat APO4-α (SEQ ID NO:14) or naturally occurring murine APO4-β (SEQ ID NO:16) or naturally occurring murine APO4-γ (SEQ ID NO:32) and is intended to include related polypeptides having substantial amino acid sequence similarity to SEQ ID NOS:8, 10, 12, 14, 16 or 32. Such related polypeptides exhibit greater sequence similarity to mAPO4-αL, mAPO4-αS, hAPO4-α, rAPO4-α, mAPO4-β or

mAPO4-γ than to other TNF family receptors and include alternatively spliced forms of human, murine or rat APO4-α, APO4-β or APO4-γ full-length forms of APO4, species homologues, and isotype variants of the amino acid sequences shown in Figures 7 and 8. As used herein, the term APO4 polypeptide describes polypeptides generally including an amino acid region with greater than about 35% amino acid sequence identity with mAPO4-αL (SEQ ID NO:8), mAPO4-αS (SEQ ID NO:10), hAPO4-α (SEQ ID NO:12), rAPO4-α (SEQ ID NO:14) or mAPO4-β (SEQ ID NO:16). In particular, an APO4 polypeptide can have greater than about 55% amino acid identity, preferably greater than about 65% amino acid identity, more preferably greater than about 75% amino acid identity, still more preferably greater than about 85% amino acid identity and most preferably greater than about 90%, 95% or 97% amino acid identity with mAPO4-αL (SEQ ID NO:8), mAPO4-αS (SEQ ID NO:10), hAPO4-α (SEQ ID NO:12), rAPO4-α (SEQ ID NO:14), mAPO4-β (SEQ ID NO:16) or mAPO4-γ (SEQ ID NO:32).

The present invention also provides active fragments of the APO4 polypeptides of the invention. As used herein, the term "active fragment of an APO4 polypeptide" is synonymous with "active fragment of APO4" or "active APO4 fragment" and means a polypeptide fragment having substantially the same amino acid sequence as a portion of an APO4-α, APO4-β or APO4-γ polypeptide, provided that the fragment retains at least one biological activity of an APO4 polypeptide. An active fragment of an APO4 polypeptide can have, for example, an amino acid sequence that is identical or substantially the same as a portion of the amino acid sequence of mAPO4-αL (SEQ ID NO:8), mAPO4-αS (SEQ ID NO:10), hAPO4-α (SEQ ID NO:12), rAPO4-α (SEQ ID NO:14), mAPO4-β (SEQ ID NO:16), or APO4-γ (SEQ ID NO:32),

provided that the fragment retains at least one biological activity of an APO4 polypeptide. A biological activity of an APO4 polypeptide can be, for example, the ability to bind an APO4 ligand, the ability to induce or suppress apoptosis, the ability to activate the JNK pathway, or the ability to induce or suppress cell proliferation, differentiation or cytokine secretion. A biological activity of an APO4 polypeptide or fragment can be routinely assayed; for example, apoptotic activity can be analyzed by transfecting an APO4 encoding nucleic acid and measuring the number of cells with apoptotic morphology, as set forth in Example II.

An active fragment of APO4 also be, for example, a cytoplasmic fragment of APO4. An active fragment of APO4 can be an N-terminal fragment having the amino terminal 355 amino acids of APO4, which was able to activate the JNK pathway in 293 EBNA cells.

An "active fragment of an APO4 polypeptide" also can be an active segment of an APO4 polypeptide. Such an active segment is a polypeptide portion having substantially the same amino acid sequence as a portion of an APO4 polypeptide, provided that the segment does not consist of the identical amino acid sequence encoded by an expressed sequence tag having GenBank accession number AA036247, AA003356, W55289, AA445805 or W56629 and provided that the segment retains at least one biological activity of an APO4 polypeptide. An active APO4 polypeptide segment can have, for example, an amino acid sequence that is identical or substantially the same as a portion of the amino acid sequence of mAPO4-αL (SEQ ID NO:8), mAPO4-αS (SEQ ID NO:10), hAPO4-α (SEQ ID NO:12), rAPO4-α (SEQ ID NO:14), mAPO4-β (SEQ ID NO:16) or APO4-γ (SEQ ID NO:32), provided that the segment does not

consist of the identical amino acid sequence encoded by an expressed sequence tag having GenBank accession number AA036247, AA003356, W55289, AA445805 or W56629 and provided that the segment retains at least one biological activity of an APO4 polypeptide.

In one embodiment, the invention provides a soluble active fragment of an APO4 polypeptide. Such a soluble active fragment includes the ligand binding domain of an APO4 polypeptide and can be, for example, a truncated polypeptide encoding the extracellular domain of an APO4 polypeptide. A soluble active fragment of an APO4 polypeptide can have, for example, substantially the same amino acid sequence as the signal peptide and ligand-binding domain of mAPO4-α (amino acids 1 to 177 of mAPO4-α shown in Figure 7A) or substantially the same sequence as mAPO4-γ (Figure 7E) or substantially the same sequence as the signal peptide and ligand-binding domain of hAPO4-α (amino acids 1 to 177 of hAPO4-α shown in Figure 7C). A specific example of such a soluble fragment is mAPO4-γ. One skilled in the art understands that a soluble active fragment of an APO4 polypeptide is distinguished from a membrane-bound form by deletion or inactivation of the transmembrane domain. The transmembrane domain of murine APO4-α is shown in Figure 7A as amino acids 173 to 191, and the transmembrane domain of human APO4-α is shown in Figure 7C as amino acids 178 to 191. A soluble active fragment of an APO4 polypeptide also can be, for example, an active segment of an APO4 polypeptide. Such active segments of APO4 polypeptides are described hereinabove.

In another embodiment, the invention provides an APO4-related polypeptide, which has a sequence with substantial similarity to the unique cytoplasmic domain

of an APO4 polypeptide. The APO4 cytoplasmic domain is a conserved region that can function to promote self-association or association with a different protein. The APO4 cytoplasmic domain also can promote ligand-dependent cell survival, proliferation, differentiation or death. An APO4-related polypeptide can be, for example, a transmembrane receptor including a cytoplasmic domain having substantial similarity to the cytoplasmic domain of mAPO4-αL or hAPO4-α. The extracellular domain of an APO4-related polypeptide receptor can be substantially the same as, or can be entirely different than the extracellular domain of an APO4 polypeptide. APO4-related polypeptides also include intracellularly expressed polypeptides that contain one or more APO4 cytoplasmic domains. An APO4 cytoplasmic domain can have, for example, the amino acid sequence shown as amino acids 192 to 416 in Figure 7A, the amino acid sequence shown as amino acids 192 to 423 in Figure 7C, or an amino acid sequence with substantial similarity to one of these sequences.

The invention further provides an isolated APO6 polypeptide having substantially the same amino acid sequence as APO6, or an active fragment thereof. An isolated APO6 polypeptide of the invention can have, for example, substantially the same amino acid sequence as the human APO6 sequence SEQ ID NO:18 shown in Figure 9.

As used herein, the term "APO6" means an APO6 polypeptide and includes polypeptides having substantially the same amino acid sequence as the hAPO6 polypeptide having amino acid sequence SEQ ID NO:18. A partial nucleotide sequence encoding hAPO6, which lacks 5' and 3' sequence, is shown in Figure 9. The available hAPO6 sequence shows about 33% homology to the

extracellular domain of TNFR-2. The partial APO6 sequence shown in Figure 9 can be part of a full-length APO6 transmembrane receptor.

The term APO6 encompasses a polypeptide having the sequence of the naturally occurring human APO6 polypeptide (SEQ ID NO:18) and is intended to include related polypeptides having substantial amino acid sequence similarity to hAPO6 (SEQ ID NO:18). Such related polypeptides exhibit greater sequence similarity to hAPO6 than to other members of the TNF receptor superfamily and include alternatively spliced forms of hAPO6, full-length forms of APO6 having a cytoplasmic signaling domain, species homologues, and isotype variants of the amino acid sequence shown in Figure 9. Such full-length forms or APO6 species homologues can be readily obtained by the skilled artisan using routine molecular techniques, for example, by screening an appropriate cDNA library with a portion of SEQ ID NO:17 as a probe. As used herein, the term APO6 describes polypeptides generally having an amino acid sequence with greater than about 40% amino acid identity with the extracellular domain of hAPO6 (SEQ ID NO:18), preferably greater than about 65% amino acid identity with the extracellular domain of hAPO6 (SEQ ID NO:18), more preferably greater than about 75% amino acid identity with the extracellular domain of hAPO6 (SEQ ID NO:18), still more preferably greater than about 75% amino acid identity with the extracellular domain of hAPO6 (SEQ ID NO:18), and most preferably about 90%, 95% or 97% amino acid identity with the extracellular domain of hAPO6 (SEQ ID NO:18).

The present invention also provides active fragments of the APO6 polypeptide of the invention. As

used herein, the term "active fragment of an APO6 polypeptide" is synonymous with "active fragment of APO6" or "active APO6 fragment" and means a polypeptide fragment having substantially the same amino acid sequence as a portion of an APO6 polypeptide, provided that the fragment retains at least one biological activity of an APO6 polypeptide. An active fragment of an APO6 polypeptide can have, for example, an amino acid sequence that is identical or substantially the same as a portion of an amino acid sequence of hAPO6 (SEQ ID NO:18), provided that the fragment retains at least one biological activity of an APO6 polypeptide. Biological activities of APO6 include the ability to bind an APO6 ligand, the ability to induce or suppress apoptosis and the ability to induce or suppress cell proliferation, differentiation or cytokine secretion. A biological activity of an APO6 polypeptide or fragment can be routinely assayed; for example, apoptotic activity can be analyzed by transfecting an APO6 encoding nucleic acid and measuring the number of cells with apoptotic morphology, as set forth in Example II.

An active fragment of an APO6 polypeptide also can be an active APO6 segment, which is a polypeptide segment having substantially the same amino acid sequence as a portion of an APO6 polypeptide, provided that the segment does not consist of the identical amino acid sequence encoded by an expressed sequence tag having GenBank accession number AA025673, AA025672, AA155701, AA155646, W67560 or AA158406 and provided that the segment retains at least one biological activity of an APO6 polypeptide. An active APO6 segment can have, for example, an amino acid sequence that is identical or substantially the same as a portion of the amino acid sequence of hAPO6 (SEQ ID NO:18), provided that the

segment does not consist of the identical amino acid sequence encoded by an expressed sequence tag having GenBank accession number AA025673, AA025672, AA155701, AA155646, W67560 or AA158406 and provided that the segment retains at least one biological activity of an APO6 polypeptide.

In one embodiment, the invention provides a soluble APO6 active fragment that includes an APO6 ligand binding domain. A soluble APO6 active fragment can be, for example, a truncated polypeptide encoding the extracellular domain of an APO6 polypeptide. A soluble APO6 active fragment can have, for example, substantially the same amino acid sequence as amino acids 1 to 168 of the hAPO6 sequence shown in Figure 1. Soluble APO6 active fragments are distinguished from membrane-bound forms by the absence of a functional transmembrane domain. A soluble APO6 active fragment also can be, for example, an active APO6 segment. Active APO6 segments are described hereinabove.

Ligands of the TNF ligand superfamily are acidic, TNF-like molecules with approximately 20% sequence homology in the extracellular receptor-binding domain. Most exist principally as type II membrane glycoproteins, with the biologically active form a trimeric or multimeric complex. Like the prototypic TNF-α molecule, most have a large C-terminal extracellular domain, a single transmembrane spanning region and a variable amino-terminal cytoplasmic domain. The TNFR ligand family includes TNF-α, TNF-β, LTβ, FAS ligand, CD27 ligand, CD30 ligand, CD40 ligand, 4-1BB ligand, OX40 ligand and FAS/APO-1 ligand. Although members of the TNF ligand superfamily typically are cell

surface-expressed molecules, soluble forms of TNF-α, TNF-β and FAS ligand also have been identified.

Disclosed herein is subfamily of polypeptide ligands designated the tumor necrosis factor related ligand 1 (TNRL1) subfamily. Thus, the present invention provides an isolated TNRL1 polypeptide having substantially the same amino acid sequence as TNRL1, or an active fragment thereof. An isolated TNRL1 polypeptide of the invention can have, for example, substantially the same amino acid sequence as the sequence of human TNRL1-α (SEQ ID NO:20); murine TNRL1-α (SEQ ID NO:22); human TNRL1-β (SEQ ID NO:24) or murine TNRL1-β (SEQ ID NO:26).

As used herein, the term "TNRL1" means a TNRL1 polypeptide and includes polypeptides having substantially the same amino acid sequence as the hTNRL1-α polypeptide having amino acid sequence SEQ ID NO:20; the mTNRL1-α polypeptide having amino acid sequence SEQ ID NO:22; the hTNRL1-β polypeptide having amino acid sequence SEQ ID NO:24; and the mTNRL1-β polypeptide having amino acid sequence SEQ ID NO:26. Human TNRL1-α (hTNRL1-α) is a polypeptide of at least 230(?) amino acids; the available hTNRL1-α nucleotide (SEQ ID NO:19) and amino acid sequence (SEQ ID NO:20) are shown in Figure 11A. Human TNRL1-α (SEQ ID NO:20) exhibits about 25% amino acid identity and about 49% amino acid similarity to TNF-α.

Murine TNRL1-α (mTNRL1-α) is the homolog of human TNRL1-α and also is an TNRL1 polypeptide of the invention. mTNRL1-α is a polypeptide of at least 290 amino acids; the available mTNRL1-α nucleotide (SEQ ID NO:21) and amino acid sequence (SEQ ID NO:22) are shown

in Figure 11B. Murine TNRL1-α (SEQ ID NO:22) is about 68% identical and 81% similar to human TNRL1-α at the amino acid level.

Human TNRL1-β (hTNRL1-β) also is a TNRL1 polypeptide of the invention. Human TNRL1-β is a polypeptide of 250 amino acids; the hTNRL1-β nucleotide sequence (SEQ ID NO:23) and amino acid sequence (SEQ ID NO:24) are shown in Figure 12A. The hTNRL1-β polypeptide (SEQ ID NO:24) is about 31% identical and 55% similar to hTNRL1-α at the amino acid level and is more distantly related to TNF-α (see Figure 10).

Murine TNRL1-β (mTNRL1-β), the homolog of human TNRL1-β, also is a TNRL1 polypeptide of the invention. The nucleotide (SEQ ID NO:25) and corresponding amino acid sequence (SEQ ID NO:26) of full-length mTNRL1-β are shown in Figure 12B.

The term TNRL1 encompasses a polypeptide having the sequence of the naturally occurring human TNRL1-α polypeptide SEQ ID NO:20, the murine TNRL1-α polypeptide SEQ ID NO:22, the human TNRL1-β polypeptide SEQ ID NO:24, or the murine TNRL1-β polypeptide SEQ ID NO:26 and is intended to include related polypeptides having substantial amino acid sequence similarity to hTNRL1-α, mTNRL1-α, hTNRL1-β or mTNRL1-β. Such related polypeptides exhibit greater sequence similarity to hTNRL1-α, mTNRL1-α, hTNRL1-β or mTNRL1-β than to other members of the TNF ligand superfamily, such as TNF-α, and include alternatively spliced forms of hTNRL1-α, full-length forms of hTNRL1-α, mTNRL1-α, hTNRL1-β or mTNRL1-β, species homologues, and isotype variants of the amino acid sequences shown in Figures 11 and 12. As used herein, the term TNRL1 describes polypeptides generally

having an amino acid sequence with greater than about 30% amino acid identity with hTNRL1-α (SEQ ID NO:20), mTNRL1-α (SEQ ID NO:22), hTNRL1-β (SEQ ID NO:24) or mTNRL1-β (SEQ ID NO:26), preferably greater than about 35% amino acid identity with hTNRL1-α (SEQ ID NO:20), mTNRL1-α (SEQ ID NO:22), hTNRL1-β (SEQ ID NO:24) or mTNRL1-β (SEQ ID NO:26), more preferably greater than about 45% amino acid identity with hTNRL1-α (SEQ ID NO:20), mTNRL1-α (SEQ ID NO:22), hTNRL1-β (SEQ ID NO:24) or mTNRL1-β (SEQ ID NO:26), and most preferably about 65%, 75%, 85%, 90%, 95% or 97% amino acid identity with hTNRL1-α (SEQ ID NO:20), mTNRL1-α (SEQ ID NO:22), hTNRL1-β (SEQ ID NO:24) or mTNRL1-β (SEQ ID NO:26).

The present invention also provides an isolated TNRL1 polypeptide having substantially the same amino acid sequence as TNRL1, or an active fragment of a TNRL1 polypeptide of the invention. An isolated TNRL1 polypeptide of the invention can have, for example, substantially the same amino acid sequence as the human TNRL1 (SEQ ID NO:20) shown in Figure 11.

The present invention also provides active fragments of a TNRL1 polypeptide of the invention. As used herein, the term "active fragment of an TNRL1 polypeptide" is synonymous with "active fragment of TNRL1" or "active TNRL1 fragment" and means a polypeptide fragment having substantially the same amino acid sequence as a portion of a TNRL1 polypeptide, provided that the fragment retains at least one biological activity of a TNRL1 polypeptide. An active fragment of a TNRL1 polypeptide can have, for example, an amino acid sequence that is identical or substantially the same as a portion of the amino acid sequence of hTNRL1-α (SEQ ID NO:20), mTNRL1-α (SEQ ID NO:22), hTNRL1-β (SEQ ID NO:24)

or mTNRL1-β (SEQ ID NO:26), provided that the fragment retains at least one biological activity of a TNRL1 polypeptide. A biological activity of a TNRL1 polypeptide can be, for example, the ability to bind a TNRL1 receptor, the ability to induce or suppress apoptosis or the ability to induce or suppress cell proliferation, differentiation or cytokine secretion. A biological activity of a TNRL1 polypeptide or fragment can be routinely assayed; for example, apoptotic activity can be analyzed by treating BJAB cells with polypeptide and assaying cell survival as set forth in Example VI.

An "active fragment of a TNRL1 polypeptide" also can be an active TNRL1 segment, which is a polypeptide segment having substantially the same amino acid sequence as a portion of a TNRL1 polypeptide, provided that the segment does not consist of the identical amino acid sequence encoded by an expressed sequence tag having GenBank accession number AA166695, T87299, R16882, AA254047, AA056924, AA057069, N91002, N79018 or AA497494 or TIGR sequence number M78231 and provided that the segment retains at least one biological activity of a TNRL1 polypeptide. An active TNRL1 segment can have, for example, an amino acid sequence that is identical or substantially the same as a portion of the amino acid sequence of hTNRL1-α (SEQ ID NO:20), mTNRL1-α (SEQ ID NO:22), hTNRL1-β (SEQ ID NO:24) or mTNRL1-β (SEQ ID NO:26), provided that the segment does not consist of the identical amino acid sequence encoded by an expressed sequence tag having GenBank accession number AA166695, T87299, R16882, AA254047, AA056924, AA057069, N91002, N79018 or AA497494 or TIGR sequence number M78231 and provided that the segment retains at least one biological activity of a TNRL1 polypeptide.

In one embodiment, the invention provides a soluble active fragment of a TNRL1 ligand of the invention, where the active fragment includes the TNRL1 receptor binding domain. A TNRL1 ligand of the invention can be, for example, a membrane-bound ligand that transduces a signal when the cell on which it is expressed contacts a cell bearing its cognate receptor or, alternatively, a soluble form of TNRL1 can be expressed extracellularly and bind a distant receptor-bearing cell. Such a soluble TNRL1 active fragment can have the activity of a full-length TNRL1 of the invention or can be an inhibitory polypeptide that opposes the biological function of full-length ligand.

Thus, the invention provides a soluble TNRL1 active fragment that includes a TNRL1 receptor binding domain. A soluble TNRL1 active fragment can be, for example, a truncated polypeptide encoding the extracellular domain of a TNRL1 polypeptide and can have, for example, an amino acid sequence that is identical or substantially the same as amino acids 22 to 225 of hTNRL1-α, amino acids 32 to 243 of mTNRL1-α, amino acids 50 to 250 of hTNRL1-β or amino acids 42 to 241 of mRNRL-β, shown in Figure 10. One skilled in the art understands that soluble TNRL1 active fragments lack a functional TNRL1 transmembrane domain. A soluble TNRL1 active fragment also can be an active TNRL1 segment. Segments having an amino acid sequence identical to the amino acid sequence encoded by an expressed sequence tag having GenBank accession number AA166695, T87299, R16882, AA254047, AA056924, AA057069, N91002, N79018 or AA497494 or TIGR sequence number M78231 are excluded from the definition of an active TNRL1 segment, as defined herein.

The present invention further provides an isolated TNRL3 polypeptide having substantially the same amino acid sequence as TNRL3, or an active fragment thereof. An isolated TNRL3 polypeptide of the invention can have, for example, substantially the same amino acid sequence as the human TNRL3 (SEQ ID NO:28) or murine TNRL3 (SEQ ID NO:30) sequence shown in Figure 13.

As used herein, the term "TNRL3" means a TNRL3 polypeptide and includes polypeptides having substantially the same amino acid sequence as the hTNRL3 polypeptide having amino acid sequence SEQ ID NO:28 or the mTNRL3 polypeptide having amino acid sequence SEQ ID NO:30. A partial nucleotide sequence encoding the 3' portion of hTNRL3 is shown in Figure 13A. The available sequence shows homology to TRAIL ligand and to other members of the TNF ligand superfamily (see Figure 10). The murine TNRL3 homolog, mTNRL3, also is a TNRL3 polypeptide of the invention. The partial nucleotide and amino acid sequence of murine TNRL3 lacks some 5' sequence; the available sequence is shown in Figure 13B.

The term TNRL3 encompasses a polypeptide having the sequence of naturally occurring human TNRL3 (SEQ ID NO:28) or murine TNRL3 (SEQ ID NO:30) and is intended to include related polypeptides having substantial amino acid sequence similarity to the human or murine TNRL3 polypeptides SEQ ID NOS:28 or 30. Such related polypeptides exhibit greater sequence similarity to hTNRL3 or mTNRL3 than to other members of the TNF ligand superfamily such as TRAIL and include alternatively spliced forms of hTNRL3 or mTNRL3, species homologues, full-length forms and isotype variants of the amino acid sequences shown in Figure 13. As used herein, the term TNRL3 describes polypeptides generally having an amino

acid sequence with greater than about 30% amino acid sequence identity with hTNRL3 or mTNRL3 (SEQ ID NO:28 or SEQ ID NO:30), preferably greater than about 35% amino acid identity with hTNRL3 or mTNRL3, more preferably greater than about 45% amino acid identity with hTNRL3 or mTNRL3 (SEQ ID NO:28 or SEQ ID NO:30), still more preferably greater than about 65% amino acid identity with hTNRL3 or mTNRL3 (SEQ ID NO:28 or SEQ ID NO:30), and most preferably about 75%, 85%, 90%, 95% or 97% amino acid identity with hTNRL3 or mTNRL3 (SEQ ID NO:28 or SEQ ID NO:30).

Further provided herein is an active fragment of a TNRL3 polypeptide of the invention. As used herein, the term "active fragment of an TNRL3 polypeptide" is synonymous with "active fragment of TNRL3" or "active TNRL3 fragment" and means a polypeptide fragment having substantially the same amino acid sequence as a portion of a TNRL3 polypeptide, provided that the fragment retains at least one biological activity of a TNRL3 polypeptide. An active fragment of a TNRL3 polypeptide can have, for example, substantially the same amino acid sequence as a portion of hTNRL3 (SEQ ID NO:28) or mTNRL3 (SEQ ID NO:30), provided that the fragment retains at least one biological activity of a TNRL3 polypeptide. A biological activity of a TNRL3 polypeptide can be, for example, the ability to bind a TNRL3 receptor, the ability to induce or suppress apoptosis or the ability to induce or suppress cell proliferation, differentiation or cytokine secretion. A biological activity of a TNRL3 polypeptide or fragment can be routinely assayed; for example, apoptotic activity can be analyzed by treating BJAB cells with polypeptide and assaying cell survival as set forth in Example VI.

An "active fragment of a TNRL3 polypeptide" also can be an active TNRL3 segment, which is a polypeptide segment having substantially the same amino acid sequence as a portion of a TNRL3 polypeptide, provided that the segment does not consist of the amino acid sequence, or a segment thereof, encoded by an expressed sequence tag having GenBank accession number R55285, N35070, C00994 or AA221610 and provided that the segment retains at least one biological activity of a TNRL3 polypeptide. An active TNRL3 segment can have, for example, an amino acid sequence that is identical or substantially the same as a portion of the amino acid sequence of hTNRL3 (SEQ ID NO:28) or mTNRL3 (SEQ ID NO:30), provided that the segment does not consist of the amino acid sequence, or a segment thereof, encoded by an expressed sequence tag having GenBank accession number R55285, N35070, C00994 or AA221610 and provided that the segment retains at least one biological activity of a TNRL3 polypeptide.

In one embodiment, the invention provides a soluble TNRL3 active fragment that includes a TNRL3 receptor binding domain. Such a soluble TNRL3 active fragment can be, for example, a truncated polypeptide encoding the extracellular domain of a TNRL3 polypeptide. Soluble TNRL3 active fragments can have, for example, an amino acid sequence that is identical or substantially the same as amino acids 4 to 208 of hTNRL3 or amino acids 7 to 211 of mTNRL3, as shown in Figure 10. Such a soluble TNRL3 active fragment lacks a functional transmembrane domain. A soluble TNRL3 active fragment also can be, for example, an active TNRL3 segment. Such active TNRL3 segments are described hereinabove.

As used herein, the term "substantially the same amino acid sequence," when used in reference to a polypeptide, fragment or segment of the invention, is intended to mean a polypeptide, fragment or segment having an identical amino acid sequence, or a polypeptide, fragment or segment having a similar, non-identical sequence that is considered by those skilled in the art to be a functionally equivalent amino acid sequence. For example, polypeptide including substantially the same amino acid sequence as APO8 can have an amino acid sequence identical to the sequence of hAPO8 (SEQ ID NO:2) shown in Figure 2, or a similar, non-identical sequence that is functionally equivalent. An amino acid sequence that is "substantially the same" can have one or more modifications such as amino acid additions or substitutions relative to the amino acid sequence shown, provided that the modified polypeptide retains substantially at least one biological activity of the polypeptide. Biological activities of the polypeptides of the invention are described herein; for example, biological activities of APO8 include the ability to bind an APO8 ligand, bind the TRADD or RIP adaptor molecules, induce or suppress apoptosis, activate NF-κB expression, or induce or suppress cell proliferation, differentiation or cytokine secretion. Comparison of sequences for substantial similarity can be performed between two sequences of any length and usually is performed with nucleotide sequences of between 5 and 3500 nucleotides, preferably between about 10 and 300 nucleotides and more preferably between about 15 and 50 nucleotides. Comparison for substantial similarity between amino acid sequences is usually performed with sequences between about 6 and 1200 residues, preferably between about 10 and 100 residues and more preferably between about 25 and 35 residues. Such comparisons for

substantial similarity are performed using methodology routine in the art.

Therefore, it is understood that limited modifications can be made without destroying the biological function of a polypeptide or ligand of the invention. For example, minor modifications of hAPO8 (SEQ ID NO:2) that do not destroy polypeptide activity also fall within the definition of APO8 and within the definition of the polypeptide claimed as such. Similarly, minor modifications of hAPO9 that do not destroy polypeptide activity fall within the definition of APO9; minor modifications of mAPO4-αL, mAPO4-αS, hAPO4-α, rAPO4-α or mAPO4-β that do not destroy polypeptide activity fall within the definition of an APO4 polypeptide; and minor modifications of hAPO6 that do not destroy polypeptide activity fall within the definition of APO6. Similarly, minor modifications of hTNRL1-α, mTNRL1-α, hTNRL1-β or mTNRL1-β that do not destroy polypeptide activity fall within the definition of TNRL1, and minor modifications of hTNRL3 and mTNRL3 that do not destroy polypeptide activity fall within the definition of TNRL3. Also, for example, genetically engineered fusion proteins that retain at least one measurable biological activity of a polypeptide of the invention fall within the definition of the polypeptides claimed as such.

It is understood that minor modifications of primary amino acid sequence can result in polypeptides which have substantially equivalent or enhanced function as compared to the APO8 polypeptide sequence set forth in Figure 2, substantially equivalent or enhanced function as compared to the hAPO9 sequence set forth in Figure 6, substantially equivalent or enhanced function as compared

to the APO4 polypeptide sequences set forth in Figures 6, 7 and 8, or substantially equivalent or enhanced function as compared to the hAPO6 sequence set forth in Figure 9. Also, minor modifications of primary amino acid sequence can result in polypeptides having substantially equivalent or enhanced function as compared to the TNRL1 polypeptide sequences shown in Figures 11 and 12, or in polypeptides having substantially equivalent or enhanced function as compared to the TNRL3 polypeptide sequences shown in Figure 13. These modifications can be deliberate, as through site-directed mutagenesis, or can be accidental such as through mutation in hosts harboring an encoding nucleic acid. All such modified polypeptides are included in the definition of an APO8 polypeptide as long as at least one biological function of APO8 is retained. Similarly, all such modified polypeptides are included in the definition of an APO9, APO4, APO6, TNRL1 or TNRL3 polypeptide as long as at least one biological function of an APO9, APO4, APO6, TNRL1 or TNRL3 polypeptide, respectively, is retained. Further, various molecules can be attached to an APO8, APO9, APO4, APO6, TNRL1 or TNRL3 polypeptide, for example, other polypeptides, carbohydrates, lipids, or chemical moieties. Such modifications are included within the definition of each of the polypeptides of the invention.

Polypeptide fragments or segments to be screened for activity can be produced, for example, by recombinant methods or by chemical or proteolytic cleavage of the isolated polypeptide. Methods for chemical and proteolytic cleavage and for purification of the resultant polypeptide fragments are well known in the art as described above. (See, for example, Deutscher, Methods in Enzymology, Vol. 182, "Guide to Protein

Purification," San Diego: Academic Press, Inc. (1990), which is incorporated herein by reference).

A polypeptide fragment or segment of the invention can be screened, for example, for pro-apoptotic or anti-apoptotic activity. Pro-apoptotic activity is the ability either alone, or in combination with another molecule, to produce cell death accompanied by at least one of the morphological or biochemical alterations characteristic of apoptosis. Morphological alterations characteristic of apoptosis are well known in the art and include, for example, condensed and rounded cellular morphology; membrane blebbing; the formation of apoptotic bodies, which are membrane-bound bodies containing cytoplasmic and nuclear components; and condensation of the nucleus, with cytoplasmic organelles being relatively well maintained (Studzinski (Ed.), Cell Growth and Apoptosis, Oxford: Oxford University Press (1995), which is incorporated herein by reference). Biochemical alterations characteristic of apoptosis also are well known in the art. The classical biochemical alteration characteristic of apoptosis is the appearance of oligonucleosome-sized fragments of DNA, which produce a "ladder" upon agarose gel electrophoresis. This extensive fragmentation can be preceded by an earlier endonucleolytic cleavage of chromatin, producing DNA fragments of about 50 kb to 300 kb in size.

A variety of assays for determining whether a polypeptide fragment or segment has pro-apoptotic or anti-apoptotic activity are well known in the art. Such methods include light microscopy for determining the presence of one or more morphological characteristics of apoptosis, such as condensed or rounded morphology, shrinking and blebbing of the cytoplasm, preservation of

structure of cellular organelles including mitochondria, and condensation and margination of chromatin. As described in Example II, cells expressing fragments of hAPO8 were determined to be apoptotic by scoring for condensed and rounded morphology.

A polypeptide fragment or segment of the invention also can be assayed for pro-apoptotic or anti-apoptotic activity using terminal deoxytransferase-mediated (TdT) dUTP biotin nick end-labeling (TUNEL) (Gavriel et al., J. Cell Biol. 119:493 (1992), which is incorporated herein by reference; Gorczyca et al., Int. J. Oncol. 1:639 (1992); Studzinski, *supra,* 1995). ApopTag™ (ONCOR, Inc., Gaithersburg, MD) is a commercially available kit for identification of apoptotic cells using digoxygenin labeling. In addition, a polypeptide fragment or segment of the invention can be assayed for pro-apoptotic or anti-apoptotic activity by detecting nucleosomal DNA fragments using agarose gel electrophoresis (Studzinski, *supra,* 1995; Gong et al., Anal. Biochem. 218:314 (1994)).

DNA filter elution methodology also can be used to detect apoptosis-associated DNA fragmentation and to determine pro-apoptotic or anti-apoptotic activity (Studzinski, *supra,* 1995; Bertrand et al., Drug Devel. 34:138 (1995), which is incorporated herein by reference). Pro-apoptotic or anti-apoptotic activity also can be detected and quantitated by determining an altered mitochondrial to nuclear DNA ratio as described in Tepper et al., Anal. Biochem. 203:127 (1992) and Tepper and Studzinski, J. Cell Biochem. 52:352 (1993), each of which is incorporated herein by reference. One skilled in the art understands that these, or other

assays for pro-apoptotic or anti-apoptotic activity, can be performed using methodology routine in the art.

A nucleic acid to be assayed can encode a polypeptide fragment or segment corresponding to a portion of a native polypeptide of the invention or can be modified to encode one or more amino acid substitutions, deletions or insertions. One or more point mutations can be introduced into the nucleic acid encoding the modified polypeptide, fragment or segment to be assayed using, for example, site-directed mutagenesis (see Wu (Ed.), Meth. In Enzymol. Vol. 217, San Diego: Academic Press (1993); Chapter 22 of Innis et al. (Ed.), PCR Protocols, San Diego: Academic Press, Inc. (1990), each of which is incorporated herein by reference). Such mutagenesis can be used to introduce a specific, desired amino acid substitution, deletion or insertion; alternatively, a nucleic acid sequence can be synthesized having random nucleotides at one or more predetermined positions to generate random amino acid substitutions. Scanning mutagenesis also can be useful in generating nucleic acid molecules encoding, for example, an APO8 polypeptide, fragment or segment that is modified throughout the entire sequence. Such a modified fragment or segment can be screened, for example, for the ability to bind an APO8 ligand; the ability to bind TRADD or RIP; the ability to induce or suppress apoptosis or the ability to activate NF-κB.

A polypeptide of the invention can be obtained by a variety of methods known within the art, including biochemical, recombinant and chemical synthesis methods. Biochemical methods for isolating a polypeptide of the invention, or active fragment thereof, include preparative gel electrophoresis, gel filtration, affinity

chromatography, ion exchange and reversed phase chromatography, chromatofocusing, isoelectric focusing and sucrose or glycerol density gradients (see, for example, Chapter 38 of Deutscher, Methods in Enzymology: Guide to Protein Purification, Vol. 182, Academic Press, Inc., San Diego (1990) and Chapter 8 of Balch et al., Methods in Enzymology, Vol. 257, Academic Press, Inc., San Diego (1995), each of which is incorporated herein by reference in its entirety). For example, as disclosed herein in Example I, human APO8 RNA is expressed in a variety of human tissues including spleen, thymus, prostate, testis, ovary, small intestine, colonic mucosa and peripheral blood lymphocyte. From these results, one skilled in the art knows that one of these tissues can be used as a source of material for isolating an hAPO8 polypeptide of the invention.

Preparative gel electrophoresis can be useful in preparing an isolated polypeptide, active fragment or active segment of the invention. For example, an APO8 polypeptide, or an active fragment or segment thereof, can be isolated by preparative polyacrylamide gel electrophoresis and elution by diffusion or electroelution (see, for example, Chapter 33 of Deutscher, *supra,* 1990). Continuous elution gel electrophoresis using a system such as the Model 491 Prep Cell (BioRad, Hercules, CA) can be used to purify a polypeptide or an active fragment or segment of the invention. If desired, continuous elution gel electrophoresis can be combined with further purification steps such as liquid phase preparative isoelectric focusing using, for example, the Rotofor system (BioRad).

Affinity chromatography is particularly useful in preparing an isolated polypeptide or an active

fragment or segment of the invention. For example, a polypeptide that interacts with an APO8 polypeptide can be useful as an affinity matrix for isolating an APO8 polypeptide or an active APO8 fragment or segment of the invention. As disclosed herein, APO8 interacts physically with TRADD and RIP (Example II), indicating that TRADD or RIP can be used as an affinity matrix for isolating an APO8 polypeptide. Death domain containing polypeptides or fragments also can be useful as an affinity matrix for isolating an APO8 polypeptide or an active APO8 fragment or segment of the invention.

Immunoaffinity chromatography can be particularly useful in isolating a polypeptide or an active fragment or segment of the invention. For example, immunoprecipitation or column chromatography with an antibody that selectively binds APO8 can be used to isolate an APO8 polypeptide or active fragment thereof. An anti-APO8 monoclonal or polyclonal antibody that selectively binds APO8 can be prepared using an immunogen such as the sequence shown as SEQ ID NO:2, or a synthetic peptide fragment thereof, as described further below. Methods of affinity chromatography are well known in the art and are described, for example, in Chapters 29, 30 and 38 of Deutscher, *supra,* 1990, which has been incorporated herein by reference.

Recombinant methods for producing a polypeptide through expression of a nucleic acid sequence in a suitable host cell also are well known in the art and are described, for example, in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed, Vols 1 to 3, Cold Spring Harbor Laboratory Press, New York (1989), which is incorporated herein by reference. A nucleic acid for expression of an APO8 polypeptide is provided herein as

SEQ ID NO:1 and a nucleic acid for expression of an APO8RP polypeptide is provided herein as SEQ ID NO:3. A nucleic acid for expression of an APO9 polypeptide is provided herein as SEQ ID NO:5; nucleic acids for expression of APO4 polypeptides are provided herein as SEQ ID NOS:7, 9, 11, 13 and 15; and a nucleic acid for expression of an APO6 polypeptide is provided herein as SEQ ID NO:17. Nucleic acids for expression of a TNRL1 polypeptide also are provided as SEQ ID NOS:19, 21, 23 and 25; and nucleic acids for expression of a TNRL3 polypeptide are provided herein as SEQ ID NOS:27 and 29.

A recombinant polypeptide of the invention, or active fragment or segment thereof, also can be expressed as a fusion protein with a heterologous "tag" for convenient isolation from bacterial or mammalian host proteins. For example, histidine-tagged recombinant APO8 can be isolated by nickel-chelate chromatography. Similarly, a glutathione-S-transferase tag or an antigenic tag such as "FLAG," "AU" or a myc epitope tag also can be included in a recombinant polypeptide or active fragment of the invention (Sambrook et al., *supra*, 1989). The PinPoint™ expression system is a commercially available system for expression of a polypeptide or active fragment as a fusion protein with a heterologous biotinylated peptide.

A polypeptide or an active fragment or segment of the invention also can be produced by chemical synthesis, for example, by the solid phase peptide synthesis method of Merrifield et al., J. Am. Chem. Soc. 85:2149 (1964), which is incorporated herein by reference. Standard solution methods well known in the art also can be used to synthesize a polypeptide or an active fragment or segment useful in the invention (see,

for example, Bodanszky, Principles of Peptide Synthesis, Springer-Verlag, Berlin (1984) and Bodanszky, Peptide Chemistry, Springer-Verlag, Berlin (1993), each of which is incorporated herein by reference). A newly synthesized polypeptide, fragment or segment can be purified, for example, by high performance liquid chromatography (HPLC) and can be characterized using mass spectrometry or amino acid sequence analysis.

Also provided herein are nucleic acid molecules encoding the tumor necrosis family receptors and ligands of the invention. These nucleic acid molecules are useful, for example, in producing recombinant polypeptides and as probes for diagnosing diseases mediated by the polypeptides of the invention, as described further below. A nucleic acid molecule of the invention can have a nucleotide sequence of, for example, about 15 to about 1500 nucleotides. In particular, a nucleic acid molecule of the invention can have a sequence of about 15, 18, 20, 25, 30, 35, 50, 100, 200, 500 or more nucleotides.

The term "isolated," as used herein in reference to a nucleic acid molecule of the invention, means a nucleic acid molecule that is in a form that is relatively free from contaminating lipids, polypeptides, unrelated nucleic acids and other cellular material normally associated with a nucleic acid molecule in a cell.

Provided herein is an isolated nucleic acid molecule containing a nucleotide sequence encoding substantially the same amino acid sequence as APO8, or an active segment thereof, provided that the nucleic acid molecule does not consist of the identical nucleotide

sequence of an expressed sequence tag having GenBank accession number AA223122 or AA232440. An isolated nucleic acid molecule of the invention can have, for example, a nucleotide sequence encoding substantially the same amino acid sequence as hAPO8 (SEQ ID NO:2), or an active segment thereof, provided that the nucleic acid molecule does not consist of the identical nucleotide sequence of an expressed sequence tag having GenBank accession number AA223122 or AA232440. An exemplary nucleic acid molecule of the invention encodes human APO8 and is provided herein as SEQ ID NO:1 (see Figure 2). In one embodiment, the invention provides an isolated nucleic acid molecule containing a nucleotide sequence encoding a soluble APO8 active segment having an APO8 ligand binding domain.

The invention further provides an isolated nucleic acid molecule containing a nucleotide sequence encoding substantially the same amino acid sequence as APO9, or an active segment thereof, provided that the nucleic acid molecule does not consist of the identical nucleotide sequence of an expressed sequence tag having GenBank accession number AA031883, AA150849, T71406 or R10995. For example, the invention provides an isolated nucleic acid molecule of the invention having a nucleotide sequence encoding substantially the same amino acid sequence as hAPO9 (SEQ ID NO:6), or an active segment thereof, provided that the nucleic acid molecule does not consist of the identical nucleotide sequence of an expressed sequence tag having GenBank accession number AA031883, AA150849, T71406 or R10995. An exemplary nucleic acid molecule of the invention encodes human APO9 and is provided herein as SEQ ID NO:5 (see Figure 6). In one embodiment, the invention provides an isolated nucleic acid molecule containing a nucleotide sequence

encoding a soluble APO9 active segment having an APO9 ligand binding domain.

In addition, the invention provides an isolated nucleic acid molecule containing a nucleotide sequence encoding substantially the same amino acid sequence as an APO4 polypeptide, or an active segment thereof, provided that the nucleic acid molecule does not consist of the identical nucleotide sequence of an expressed sequence tag having GenBank accession number AA036247, AA003356, W55289, AA445805 or W56629. Such a nucleic acid molecule can have a nucleotide sequence encoding substantially the same amino acid sequence as mAPO4-αL (SEQ ID NO:8), mAPO4-αS (SEQ ID NO:10), hAPO4-α (SEQ ID NO:12), rAPO4-α (SEQ ID NO:14), mAPO4-β (SEQ ID NO:16), or mAPO4-γ (SEQ ID NO:32), or an active segment thereof, provided that the nucleic acid molecule does not consist of the identical nucleotide sequence of an expressed sequence tag having GenBank accession number AA036247, AA003356, W55289, AA445805 or W56629. Exemplary nucleic acid molecules encoding the APO4 polypeptides of the invention are provided herein. As shown in Figure 7A-E, SEQ ID NO:7 is a nucleotide sequence encoding murine APO4-αL; SEQ ID NO:9 is a nucleotide sequence encoding murine APO4-αS; SEQ ID NO:11 is a nucleotide sequence encoding human APO4-α; and SEQ ID NO:13 is a nucleotide sequence encoding rat APO4-α and SEQ ID NO:31 is a nucleotide sequence encoding murine APO4-γ. Figure 8 shows SEQ ID NO:15, which is a nucleotide sequence encoding murine APO4-β. The invention also provides an isolated nucleic acid molecule containing a nucleotide sequence encoding a soluble active segment of an APO4 polypeptide having an APO4 ligand binding domain.

Also provided by the invention is an isolated nucleic acid molecule containing a nucleotide sequence encoding substantially the same amino acid sequence as APO6, or an active segment thereof, provided that the nucleic acid molecule does not consist of the identical nucleotide sequence of an expressed sequence tag having GenBank accession number AA025673, AA025672, AA155701, AA155646, W67560 or AA158406. For example, the invention provides an isolated nucleic acid molecule of the invention having a nucleotide sequence encoding substantially the same amino acid sequence as hAPO6 (SEQ ID NO:18), or an active segment thereof, provided that the nucleic acid molecule does not consist of the identical nucleotide sequence of an expressed sequence tag having GenBank accession number AA025673, AA025672, AA155701, AA155646, W67560 or AA158406. Provided in Figure 9 is an exemplary nucleic acid molecule of the invention, SEQ ID NO:17, which encodes human APO6. In one embodiment, the invention provides an isolated nucleic acid molecule containing a nucleotide sequence encoding a soluble APO6 active segment having an APO6 ligand binding domain.

The invention also provides an isolated nucleic acid molecule containing a nucleotide sequence encoding substantially the same amino acid sequence as a TNRL1 polypeptide, or an active segment thereof, provided that the nucleic acid molecule does not consist of the identical nucleotide sequence of an expressed sequence tag having GenBank accession number AA166695, T87299, R16882, AA254047, AA056924, AA057069, N91002, N79018 or AA497494 or TIGR sequence number M78231. The invention provides, for example, an isolated nucleic acid molecule having a nucleotide sequence encoding substantially the same amino acid sequence as hTNRL1-α (SEQ ID NO:20),

mTNRL1-α (SEQ ID NO:22), hTNRL1-β (SEQ ID NO:24) or mTNRL1-β (SEQ ID NO:26) or an active segment thereof, provided that the nucleic acid molecule does not consist of the identical nucleotide sequence, or a segment thereof, of an expressed sequence tag having GenBank accession number AA166695, T87299, R16882, AA254047, AA056924, AA057069, N91002, N79018 or AA497494 or TIGR sequence number M78231. Provided in Figures 11 and 12 are exemplary nucleic acid molecules of the invention: SEQ ID NO:19 encodes human TNRL1-α; SEQ ID NO:21 encodes murine TNRL1-α; SEQ ID NO:23 encodes human TNRL1-β; and SEQ ID NO:25 encodes murine TNRL1-β. The invention also provides an isolated nucleic acid molecule containing a nucleotide sequence encoding a soluble TNRL1 active segment having a TNRL1 ligand binding domain.

The invention further provides an isolated nucleic acid molecule containing a nucleotide sequence encoding substantially the same amino acid sequence as TNRL3, or an active segment thereof, provided that the nucleic acid molecule does not consist of the identical nucleotide sequence of an expressed sequence tag having GenBank accession number R55285, N35070, C00994 or AA221610. For example, the invention provides an isolated nucleic acid molecule of the invention having a nucleotide sequence encoding substantially the same amino acid sequence as hTNRL3 (SEQ ID NO:28) or mTNRL3 (SEQ ID NO:30), or an active segment thereof, provided that the nucleic acid molecule does not consist of the identical nucleotide sequence of an expressed sequence tag having GenBank accession number R55285, N35070, C00994 or AA221610. Provided in Figure 13 are exemplary nucleic acid molecules of the invention. SEQ ID NO:27 encodes human TNRL3, and SEQ ID NO:29 encodes murine TNRL3. The invention also provides an isolated nucleic acid molecule

containing a nucleotide sequence encoding a soluble TNRL3 active segment having a TNRL3 ligand binding domain.

The invention also provides selective binding agents, which are agents that exhibit selective binding for a receptor or ligand of the invention.

Provided herein are APO8 selective binding agents, which are agents that selectively bind an APO8 polypeptide or an active fragment thereof. Such an APO8 selective binding agent exhibits selective binding affinity for an APO8 polypeptide, such as a polypeptide having substantially the same amino acid sequence as hAPO8 (SEQ ID NO:2) or active fragment thereof. In addition to exhibiting selective binding affinity for APO8, such APO8 selective binding agents can also function to selectively enhance or inhibit the function of APO8. For example, an APO8 selective binding agent can function to enhance or inhibit binding of an APO8 polypeptide to its natural APO8 ligand, enhance or inhibit binding to TRADD or RIP, enhance or inhibit apoptotic activity, enhance or inhibit the capacity of APO8 to activate NF-κB, or enhance or inhibit the ability of an APO8 polypeptide to effect cell proliferation, differentiation or cytokine secretion.

The invention also provides APO9 selective binding agents, which are agents that selectively bind an APO9 polypeptide or active fragment thereof, such as a polypeptide having, for example, substantially the same amino acid sequence as hAPO9 (SEQ ID NO:6). Such APO9 selective binding agents exhibit selective binding affinity for APO9 and, in addition, can function to selectively enhance or inhibit the function of APO9. An APO9 selective binding agent can, for example, enhance or

inhibit APO9 binding to a ligand, enhance or inhibit the pro-apoptotic or anti-apoptotic activity of an APO9 polypeptide, or enhance or inhibit the ability of an APO9 polypeptide to effect cell proliferation, differentiation or cytokine secretion.

Further provided herein are APO4 selective binding agents, which are agents that selectively bind an APO4 polypeptide or active fragment thereof. An APO4 selective binding agent can exhibit selective binding affinity for an APO4 polypeptide having, for example, substantially the same amino acid sequence as mAPO4-αL (SEQ ID NO:8), mAPO4-αS (SEQ ID NO:10), hAPO4-α (SEQ ID NO:12), rAPO4-α (SEQ ID NO:14), mAPO4-β (SEQ ID NO:16) or mAPO4-γ (SEQ ID NO:32), or an active fragment thereof. Such APO4 selective binding agents exhibit selective binding affinity for an APO4 polypeptide and, in addition, can selectively enhance or inhibit the function of an APO4 polypeptide. An APO4 selective binding agent can, for example, enhance or inhibit binding of an APO4 polypeptide to a ligand, enhance or inhibit pro-apoptotic or anti-apoptotic activity of an APO4 polypeptide, or enhance or inhibit the ability of an APO4 polypeptide to effect cell proliferation, differentiation or cytokine secretion.

APO6 selective binding agents also are provided by the present invention. Such APO6 selective binding agents are agents that selectively bind an APO6 polypeptide of the invention or active fragment thereof. An APO6 selective binding agent can exhibit selective binding affinity for an APO6 polypeptide having, for example, substantially the same amino acid sequence as hAPO6 (SEQ ID NO:18), or active fragment thereof. Such APO6 selective binding agents exhibit selective binding

affinity for an APO6 polypeptide and, in addition, can selectively enhance or inhibit the function of an APO6 polypeptide. For example, an APO6 selective binding agent can enhance or inhibit the binding of an APO6 polypeptide to a ligand, enhance or inhibit the pro-apoptotic or anti-apoptotic activity of an APO6 polypeptide, or enhance or inhibit the ability of an APO6 polypeptide to effect cell proliferation, differentiation or cytokine secretion.

The invention additionally provides binding agents that selectively bind a ligand of the invention. Provided herein are TNRL1 selective binding agents, which are agents that selectively bind a TNRL1 polypeptide or active fragment thereof. A TNRL1 selective binding agent can exhibit selective binding affinity for a TNRL1 polypeptide having, for example, substantially the same amino acid sequence as hTNRL1-α (SEQ ID NO:20), mTNRL1-α (SEQ ID NO:22), hTNRL1-β (SEQ ID NO:24) or mTNRL1-β (SEQ ID NO:26), or active fragments thereof. Such TNRL1 selective binding agents exhibit selective binding affinity for a TNRL1 polypeptide and, in addition, can selectively enhance or inhibit the function of a TNRL1 polypeptide. For example, a TNRL1 selective binding agent can enhance or inhibit the binding of a TNRL1 polypeptide to its cognate receptor.

The invention further provides a TNRL3 selective binding agent, which is an agent that selectively binds a TNRL3 polypeptide or active fragment thereof. A TNRL3 selective binding agent can exhibit selective binding affinity for a TNRL3 polypeptide having, for example, substantially the same amino acid sequence as hTNRL3 (SEQ ID NO:28) or mTNRL3 (SEQ ID NO:30), or active fragments thereof. Such TNRL3

selective binding agents exhibit selective binding affinity for a TNRL3 polypeptide and, additionally, can selectively enhance or inhibit the function of a TNRL3 polypeptide. For example, a TNRL3 selective binding agent can enhance or inhibit the binding of a TNRL3 polypeptide to a cognate receptor.

The term "selective binding agent," as used herein, means an agent that exhibits selective binding to the indicated polypeptide or polypeptide family. As used herein, the term "agent" encompasses simple or complex organic molecules, peptides, peptidomimetics, polypeptides and antibodies as well as nucleic acids, carbohydrates or lipids. A selective binding agent is any such agent that binds with substantially higher affinity to the indicated polypeptide or nucleic acid than to an unrelated polypeptide or nucleic acid.

Selective binding agents include natural ligands as well as other agents that exhibit selective binding for the indicated polypeptide of the invention. Such agents include polyclonal or monoclonal antibodies as well as selective binding agents isolated, for example, from random bacteriophage libraries or combinatorial libraries. Particularly useful selective binding agents include activating antibodies that selectively bind an APO8, APO9, APO4, APO6, TNRL1 or TNRL3 polypeptide of the invention. Such activating antibodies can, for example, function to cross-link receptors and trigger receptor signaling in the absence of ligand. Particularly useful selective binding agents also include neutralizing antibodies, which can bind and dampen or inactivate the function of an APO8, APO9, APO4, APO6, TNRL1 or TNRL3 polypeptide. Particularly useful selective binding agents that selectively bind a TNRL1 or

TNRL3 polypeptide of the invention also include soluble or full-length cognate receptors, which bind their respective TNRL1 or TNRL3 polypeptide ligand.

The term "selective binding," as used in reference to a selective binding agent and an indicated polypeptide, means that the agent binds with substantially higher affinity to the indicated polypeptide, or fragment thereof, than to another polypeptide. For example, an APO8 selective binding agent, which selectively binds an APO8 polypeptide, binds with substantially higher affinity to an APO8 polypeptide, or fragment thereof, than to another member of the TNFR superfamily such as TNFR-1 or another death domain containing polypeptide such as FADD. Thus, for example, an agent that binds the death domains of TNFR-1, FAS/APO-1 and APO8 with similar affinity is not a selective binding agent as defined herein. However, an agent that has substantially higher affinity for an APO8 death domain than for the TNFR-1 and FAS/APO-1 death domains exhibits selective binding for APO8 and, therefore, is encompassed within the definition of an APO8 selective binding agent. Similarly, a TNRL1 selective binding agent, which as defined herein is an agent that selectively binds a TNRL1 polypeptide, binds with substantially higher affinity to a TNRL1 polypeptide than to another member of the TNF family such as TNF-α.

The invention also provides a method of identifying an APO8 selective binding agent by contacting an APO8 polypeptide, or fragment thereof, with an agent and determining selective binding of the agent to the APO8 polypeptide, where said the selective binding indicates that the agent is an APO8 selective binding agent.

A variety of methods for determining the selective binding of an agent to a polypeptide or fragment of the invention are well known in the art. Such methods include, for example, ELISA, RIA, receptor/ligand binding assays and other affinity assays such as chromatographic methods and panning. These screening methods are well known in the art and are described, for example in Harlow et al., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York (1992); Ansubel et al., Current Protocols in Molecular Biology, John Wiley and Sons, Baltimore, MD (1989) and in Devlin, T.M., Textbook of Biochemistry with Clinical Correlations, Wiley-Liss, New York, NY (1992).

Screening assays for detecting selective binding also include two-hybrid systems such as the yeast two hybrid system, which can be used to screen a panel of agents to detect selective binding to a polypeptide of the invention or fragment thereof (see Fields and Song, Nature 340:245-246 (1989), which is incorporated herein by reference) or a two-hybrid system adapted for use in mammalian cells (see Fearon et al., Proc. Natl. Acad. Sci., USA 89:7958-7962 (1992), which is incorporated herein by reference).

Using one of the assays described above, or another assay well known in the art, a large collection, or library, of random agents or agents or interest can be screened for selective binding activity. Polypeptide libraries and tagged chemical libraries including those comprising polypeptides, peptides and peptidomimetic molecules can be screened. Polypeptide libraries also include those generated by phage display technology. Phage display technology includes the expression of polypeptide molecules on the surface of phage as well as

other methodologies by which a protein ligand is or can be associated with the nucleic acid which encodes it. Methods for production of phage display libraries, including vectors and methods of diversifying the population of peptides which are expressed, are well known in the art (see, for example, Smith and Scott, Methods Enzymol. 217:228-257 (1993); Scott and Smith, Science 249:386-390 (1990); and Huse, WO 91/07141 and WO 91/07149, each of which is incorporated herein by reference). These or other well known methods can be used to produce a phage display library which can be screened, for example, to identify a selective binding agent of the invention.

Each of the isolated receptor polypeptides provided herein are useful as immunogens for preparing an antibodies. Thus, the invention provides an antibody that selectively binds an APO8 polypeptide, for example, hAPO8 (SEQ ID NO:2). Such an antibody is useful, for example, in purifying an APO8 polypeptide by immunoaffinity chromatography.

The invention further provides an antibody that selectively binds an APO9 polypeptide of the invention, for example, hAPO9 (SEQ ID NO:6). Similarly, the invention provides an antibody that selectively binds an APO4 polypeptide; such an antibody can selectively bind, for example, mAPO4-αL (SEQ ID NO:8), mAPO4-αS (SEQ ID NO:10), hAPO4-α(SEQ ID NO:12), rAPO4-α (SEQ ID NO:14), mAPO4-β (SEQ ID NO:16) or mAPO4-γ (SEQ ID NO:32). Further provided by the invention is an antibody that selectively binds an APO6 polypeptide, for example, hAPO6 (SEQ ID NO:18). Each of these antibodies are useful in purifying the polypeptide to which it selectively binds using immunoaffinity chromatography.

The isolated ligand polypeptides provided herein also are useful as immunogens for preparing antibodies. Thus, the invention provides antibodies that selectively bind a ligand of the invention. Provided herein is an antibody that selectively binds a TNRL1 polypeptide, for example, hTNRL1-α (SEQ ID NO:20), mTNRL1-α (SEQ ID NO:22), hTNRL1-β (SEQ ID NO:24) or mTNRL1-β (SEQ ID NO:26) or an active fragment thereof. Also provided herein is an antibody that selectively binds a TNRL3 polypeptide of the invention. Such an antibody can selectively bind, for example, hTNRL3 (SEQ ID NO:28) or mTNRL3 (SEQ ID NO:30).

As used herein, the term "antibody" is used in its broadest sense to include polyclonal and monoclonal antibodies, as well as polypeptide fragments of antibodies that retain a selective binding activity for a polypeptide of the invention of at least about 1×10^5 M^{-1}. One skilled in the art would know that antibody fragments such as Fab, $F(ab')_2$ and Fv fragments can retain selective binding activity for a polypeptide of the invention and, thus, are included within the definition of an antibody. In addition, the term antibody as used herein includes naturally occurring antibodies, as well as non-naturally occurring antibodies and fragments such as chimeric antibodies and humanized antibodies that have selective binding activity. Such non-naturally occurring antibodies can be constructed using solid phase peptide synthesis, produced recombinantly or obtained, for example, by screening combinatorial libraries consisting of variable heavy chains and variable light chains as described by Borrebaeck (Ed.), Antibody Engineering (Second edition) New York: Oxford University Press (1995), which is incorporated herein by reference.

Methods for producing antibodies are routine in the art. A purified polypeptide of the invention, which can be prepared from natural sources or produced recombinantly as described above, or a fragment thereof, such as a synthetic peptide, can be used as an immunogen. Non-immunogenic fragments or synthetic peptides can be made immunogenic by coupling the hapten to a carrier molecule such as bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH). In addition, various other carrier molecules and methods for coupling a hapten to a carrier molecule are well known in the art as described, for example, by Harlow and Lane, Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory Press, 1988), which is incorporated herein by reference.

An antibody "having selective binding for" a polypeptide, or that "selectively binds" a polypeptide, binds with substantially higher affinity to that polypeptide than to another polypeptide.

The APO8 encoding nucleic acids and polypeptides of the invention can be used to diagnose, treat or reduce the severity of an APO8-mediated disease. For example, the APO8 encoding nucleic acids, polypeptides and active fragments and segments thereof can be used for diagnosis of an APO8-mediated disease or can be used to generate reagents useful for such diagnosis. Diagnosis can be performed, for example, by nucleic acid probe hybridization, or by amplification of APO8 encoding nucleotide sequences and subsequent electrophoretic analysis such as DNA sequencing. Diagnosis also can be performed, for example, using antibody or ligand based detection with an APO8 selective binding agent. Detection can be performed *ex vivo*, for example, by removing a cell or tissue sample from an

individual at risk of, suspected of having or exhibiting one or more symptoms of an APO8-mediated disease. Altered APO8 expression or activity is indicative of an APO8-mediated disease. As used herein, the term "APO8-mediated disease" means a disease, pathology, or other condition resulting from an abnormality in the expression or activity of an APO8 polypeptide. Included within the meaning of the term "APO8-mediated disease" are disorders of cell loss, disorders of cell accumulation and disorders of differentiation, as described further below.

The present invention provides a method of treating or reducing the severity of an APO8-mediated disease in a subject by administering an APO8 polypeptide or active fragment thereof or by administering a nucleic acid molecule encoding the APO8 polypeptide or fragment. An APO8 polypeptide or nucleic acid molecule useful in such a method can be or encode, for example, an amino acid sequence that is identical or substantially the same as hAPO8 (SEQ ID NO:2), or an active fragment thereof. An APO8 antisense nucleic acid molecule, which has a nucleotide sequence that is identically or substantially complementary to an APO8 encoding nucleic acid or active fragment thereof, also can be used to treat or reduce the severity of an APO8-mediated disease according to a method of the invention.

Also provided herein is a method of treating or reducing the severity of an APO8-mediated disease in a subject by administering an APO8 regulatory agent to the subject. As used herein, an "APO8 regulatory agent" is an agent that inhibits or enhances a biological activity of an APO8 polypeptide. Such an APO8 regulatory agent can be, for example, an APO8 inhibitory agent such as a

dominant negative form of APO8, an APO8 selective binding agent that inhibits a biological activity of an APO8 polypeptide, or a cysteine protease inhibitor. Dominant negative forms of APO8 include, for example, soluble forms of APO8 having an APO8 ligand binding domain, as well as membrane bound forms of APO8 having an APO8 ligand binding domain but lacking a functional cytoplasmic tail, known as "decoy receptors." Examples of such "decoy receptor" APO8 inhibitory agents are provided herein as APO8ΔCP and APO8ΔDD (see Example II).

An APO8 regulatory agent also can be an agent that inhibits or enhances the activity or expression of FADD, FLICE, TRADD or RIP or that inhibits or enhances the activity or expression of NF-κB. One skilled in the art understands that such an APO8 regulatory agent can be an agent that selectively regulates a biological activity of an APO8 polypeptide or, alternatively, can be a non-selective agent that, in addition to regulating a biological activity of an APO8 polypeptide, also regulates the activity of, for example, other death domain containing receptors. As shown in Example II, the FADD dominant negative construct DN-FADD, the FLICE dominant negative construct (FL-C360S) and the cysteine protease inhibitors CrmA and z-VAD-fmk each inhibit APO8 induced apoptosis. In addition, the TRADD dominant negative construct ND-TRADD inhibits APO8-mediated NF-κB activation, which can protect cells from apoptosis (Example II). Thus, each of these molecules are examples of APO8 regulatory agents as defined herein.

As described above, an agent can be a simple or complex organic molecule, peptide, peptidomimetic, polypeptide, antibody, nucleic acid, carbohydrate or lipid molecule.

The present invention also provides a method of treating or reducing the severity of an APO8RP-mediated disease in a subject by administering to the subject a FADD regulatory agent. Such a FADD regulatory agent is an agent, such as a simple or complex organic molecule, peptide, peptidomimetic, polypeptide, antibody, nucleic acid, carbohydrate or lipid molecule, that inhibits or enhances FADD activity or expression. As disclosed herein, hAPO8 (SEQ ID NO:2) and hAPO8RP (SEQ ID NO:4) induce apoptosis through the FADD adaptor molecule. The data shown in Figure 5B demonstrate that a FADD dominant negative construct (DN-FD) reduces APO8RP-mediated apoptosis. These results indicate that a FADD regulatory agent, such as a FADD dominant negative construct, can be used to reduce APO8RP-induced apoptosis and, thus, treat or reduce the severity of an APO8RP-mediated disease. An "APO8 related polypeptide-mediated disease," as used herein, is synonymous with "APO8RP-mediated disease" and means a disease, pathology, or other condition resulting from an abnormality in the expression or activity of an APO8RP polypeptide and can be, for example, a disorder of cell loss, disorder of cell accumulation or disorder of cell differentiation. An APO8RP-mediated disease also can be, for example, an autoimmune disorder.

Further provided by the present invention is a method of treating or reducing the severity of an APO8RP-mediated disease in a subject by administering to the subject a TRADD regulatory agent or a RIP regulatory agent. Such an agent enhances or inhibits the activity or expression of TRADD or RIP. As disclosed herein in Example II, both APO8 and APO8RP directly interact with the TRADD and RIP adaptor molecules. Example II further demonstrates that a TRADD dominant negative construct blocks NF-κB activation by APO8 and APO8RP. Thus, a

TRADD regulatory agent or a RIP regulatory agent, such as a TRADD dominant negative construct, can be used to treat or reduce the severity of an APO8RP-mediated disease.

The present invention also provides a method of treating or reducing the severity of an APO8RP-mediated disease in a subject by administering to the subject an NF-κB regulatory agent, which is an agent that enhances or inhibits the activity or expression of NF-κB. Activation of the NF-κB pathway is known to protect cells from apoptosis, and, as disclosed herein, expression of APO8 or APO8RP results in NF-κB activation (see Example II). Thus, an NF-κB regulatory agent can block, for example, apoptosis and, therefore, can be useful in treating or reducing the severity of an APO8RP-mediated disease that results from excessive cell death.

The APO9 encoding nucleic acids and polypeptides of the invention can be used to diagnose, treat or reduce the severity of an APO9-mediated disease. For example, the APO9 encoding nucleic acids, polypeptides and active fragments thereof can be used for diagnosis of an APO9-mediated disease or can be used to generate reagents useful for such diagnosis. Diagnosis can be performed, for example, by nucleic acid probe hybridization, or by amplification of APO9 encoding nucleotide sequences and subsequent electrophoretic analysis such as DNA sequencing. Diagnosis also can be performed using, for example, antibody or ligand based detection with an APO9 selective binding agent. Detection can be performed *ex vivo*, for example, by removing a cell or tissue sample from an individual at risk of, suspected of having or exhibiting one or more symptoms of an APO8-mediated disease. Altered APO9 expression or activity is indicative of an APO9-mediated

disease. As used herein, the term "APO9-mediated disease" means a disease, pathology, or other condition resulting from an abnormality in the expression or activity of an APO9 polypeptide. Included within the meaning of the term "APO9-mediated disease" are disorders of cell loss, disorders of cell accumulation and disorders of cell differentiation.

The present invention provides a method of treating or reducing the severity of an APO9-mediated disease in a subject by administering an APO9 polypeptide or active fragment thereof or by administering a nucleic acid molecule encoding the APO9 polypeptide or fragment. An APO9 polypeptide or nucleic acid molecule useful in such a method can be or encode, for example, an amino acid sequence identical or substantially the same as hAPO9 (SEQ ID NO:6), or an active fragment thereof. An APO9 antisense nucleic acid molecule, which has a nucleotide sequence that is identically or substantially complementary to an APO9 encoding nucleic acid or active fragment thereof, also can be used to treat or reduce the severity of an APO9-mediated disease according to a method of the invention.

Further provided herein is a method of treating or reducing the severity of an APO9-mediated disease in a subject by administering an APO9 regulatory agent to the subject. As used herein, an "APO9 regulatory agent" is an agent that inhibits or enhances a biological activity of an APO9 polypeptide. Such an APO9 regulatory agent can be, for example, an APO9 inhibitory agent such as a dominant negative form of APO9, an alternative spliced full-length form of APO9, or an APO9 selective binding agent that inhibits a biological activity of an APO9 polypeptide. Dominant negative forms of APO9 include,

for example, soluble forms of APO9 having an APO9 ligand binding domain, as well as membrane bound forms of APO9 having an APO9 ligand binding domain but lacking a functional cytoplasmic tail, known as "decoy receptors." An APO9 regulatory agent can also be an agent that selectively binds an alternative spliced full-length form of APO9, thereby blocking or transmitting a signal through full-length APO9. One skilled in the art understands that such an APO9 regulatory agent can be an agent that selectively regulates a biological activity of an APO9 polypeptide or, alternatively, can be a non-selective agent that, in addition to regulating a biological activity of an APO9 polypeptide, also regulates the activity of, for example, other TNF family receptors.

The APO4 encoding nucleic acids and polypeptides of the invention can be used to diagnose, treat or reduce the severity of an APO4-mediated disease. The APO4 encoding nucleic acids, polypeptides and active fragments thereof can be used for diagnosis of an APO4-mediated disease or can be used to generate reagents useful for such diagnosis. Diagnosis can be performed, for example, by nucleic acid probe hybridization, or by amplification of APO4 encoding nucleotide sequences and subsequent electrophoretic analysis such as DNA sequencing. Diagnosis also can be performed, for example, using antibody or ligand based detection with an APO4 selective binding agent. Detection can be performed *ex vivo*, for example, by removing a cell or tissue sample from an individual at risk of, suspected of having or exhibiting one or more symptoms of an APO4-mediated disease. Altered APO4 polypeptide expression or activity can be indicative of an APO4-mediated disease. As used herein, the term "APO4-mediated disease" means a disease,

pathology, or other condition resulting from an abnormality in the expression or activity of an APO4 polypeptide. Included within the meaning of the term "APO4-mediated disease" are disorders of cell loss, disorders of cell accumulation and disorders of cell differentiation.

As disclosed herein, APO4 was expressed highly in embryonic tissue at day 11, 15 and 17, indicating that APO4 can play a role in early development (see Example IV). APO4 expression in embryonic tissues also indicates that APO4 can play a role in developmental abnormalities or in gestation. Thus, APO4 and can be used in the diagnosis or treatment of developmental or gestational abnormalities.

The present invention provides, for example, a method a diagnosing a developmental abnormality caused by the aberrant expression of APO4 during development by determining the level or pattern of expression of APO4 mRNA or protein in embryonic cells or tissue. The invention further provides a method of diagnosing a developmental abnormality casued by the aberrant expression of APO4 during development by detecting a mutation in an APO4 gene in a cell or tissue derived from embryos.

In adult tissues, APO4 expression was highest in adult prostate, with lower levels of expression seen in adult spleen, thymus, testis, uterus, small intestine, colon and peripheral blood leukocytes. APO4 also was highly expressed in a prostate carcinoma epithelial cell line, LNCaP (see Example IV). Since the glandular epithelial cells of the prostate represent the site of origin of prostate cancer and the cell type that

increases during prostate cancer growth, increased APO4 levels can be used as a marker diagnostic of the presence of prostate cancer. For example, as the levels of APO4 secreted by prostatic tissue increase with the increasing mass of the prostate epithelial cells, elevated levels of APO4 either in the serum or from a tissue biopsy of an individual suspected of having prostate cancer can be used to diagnose the existence of prostate cancer. In addition, altered APO4 levels can be assayed in an individual with prostate cancer as a method of staging the degree or severity of the cancer, thereby providing critical information for determining the most advantageous therapeutic strategy. Altered APO4 levels also can be useful for the diagnosis of local and distant metastatic spread of prostate cancer and for assessment of disease volume. Such a diagnostic indicator is particularly useful in planning radiation therapy for prostate cancer by defining the limits of the spread of disease.

Thus, the present invention provides a method of diagnosing prostate cancer in an individual by determining the level of APO4 in the individual. A method of the invention can be particularly useful in diagnosing prostate cancer in an individual at risk for this cancer, such as in men over the age of fifty or with a family history of prostate cancer.

Soluble APO4 can be a naturally occurring form secreted into the bloodstream. For example, murine APO4 form occurs naturally in soluble form and is secreted into the bloodstream. In one embodiment, the invention provides a method of diagnosing prostate cancer in an individual by determining the level of soluble APO4 in the individual. Such methods can be particularly useful

since a non-invasive blood test can be used in order to determine the level of soluble APO4.

A variety of methods can be used to assay for the level of APO4 according to a method of the invention for diagnosing prostate cancer. Such methods include assays for APO4 RNA levels, for example, reverse transcriptase polymerase chain reaction (RT-PCR) analysis. Such methods further include immunoassays for determining the level of APO4 polypeptide, for example, radioimmunoassays and enzyme-linked immunoassays. The level of APO4 in an individual suspected of having prostate cancer can be determined, for example, in a sample such as a serum sample from the individual. If desired, the level of APO4 also can be assayed *in situ*, for example, by administering an anti-APO4 antibody linked to a detectable moiety and subsequent detection of the moiety.

The invention also provides a method of identifying an effective pharmacological agent useful in the diagnosis or treatment of a disease associated with APO4 activity. The method includes the steps of contacting an APO4 polypeptide or active fragment thereof with an agent; and determining selective binding of the agent to the APO4 polypeptide or active fragment thereof, where the selective binding indicates that the agent is an effective pharmacological agent useful in the diagnosis or treatment of a disease associated with APO4 activity. An APO4 polypeptide or active fragment thereof useful in the invention can be, for example, an APO4 extracellular ligand binding domain. In one embodiment, the APO4 polypeptide or active fragment thereof is expressed in a cell or on the surface of a cell. In another embodiment, the APO4 polypeptide

or active fragment thereof is contacted with an agent *in vitro*.

Methods for determining selective binding are known in the art and are described hereinabove. For example, assays to determine selective binding include, two-hybrid systems, ELISA, RIA, receptor/ligand binding assays and other affinity assays such as chromatographic methods and panning.

The present invention also provides a method of screening for an APO4 agonist useful in treating prostate cancer. The method includes the steps of contacting a cell expressing an APO4 polypeptide or active fragment thereof with an agent; and assaying for increased APO4 activity, where increased APO4 activity indicates that the agent is an APO4 agonist useful in treating prostate cancer.

An APO4 activity can be, for example, APO4 binding to a ligand, activation of the JNK pathway or activation of NF-κB activity. APO4 activity also includes apoptotic activity in a cell in response to increased APO4 expression or contact with APO4. The stimulation or induction of apoptosis in the cell includes any increased level of apoptosis compared to the level observed prior to contact with the APO4 agonist.

Further provided herein is a method of screening for an APO4 antagonist. The method includes the steps of contacting a cell expressing an APO4 polypeptide or active fragment thereof with an agent; and assaying for decreased APO4 activity, where decreased APO4 activity indicates that the agent is an APO4 antagonist.

The invention also provides a method of identifying an effective pharmacological agent useful in the diagnosis or treatment of a disease associated with APO4 activity by forming a mixture including an APO4 polypeptide or active fragment thereof, an APO4 signal transducer molecule that specifically interacts with a cytoplasmic domain of the APO4 polypeptide or active fragment thereof, and an agent; and detecting the level of APO4 activity in the presence of the agent, where an alteration in the APO4 activity relative to control activity indicates that the agent is an effective pharmacological agent useful in the diagnosis or treatment of a disease associated with APO4 activity. An alteration in APO4 activity can be an increased or decreased activity. Examples of APO4 activity include, for example, activation of the JNK pathway, activation of NF-κB, apoptosis, cell proliferation or cell differentiation. In such a method of the invention, the APO4 activity can be measured, for example, *in vivo* or *in vitro*.

As discussed above, APO4 can be highly expressed in prostate cancer epithelial cells. Based on this expression, the present invention also provides a method of treating prostate cancer by administering to an individual having prostate cancer a conjugate including an APO4 selective binding agent linked to a therapeutic moiety. Administration of a conjugate including an APO4 selective binding agent, for example, an anti-APO4 monoclonal antibody, facilitates targeted delivery of a linked therapeutic moiety such as doxorubicin to prostate cancer epithelial cells.

As used herein, the term "APO4 selective binding agent" means a simple or complex organic

molecule, peptide, peptidomimetic, polypeptide, antibody, nucleic acid, carbohydrate or lipid that binds with substantially higher affinity to an APO4 polypeptide than to an unrelated polypeptide. Methods for identifying a selective binding agent are described hereinabove.

As disclosed herein, a therapeutic moiety can be, for example, a cancer chemotherapeutic agent linked to an APO4 selective binding agent to produce a conjugate containing an APO4 selective binding agent linked to a therapeutic moiety. Cytotoxic chemotherapy is the basis of the systemic treatment of disseminated malignant tumors. However, a major limitation of the currently used chemotherapeutic agents is that these drugs have the narrowest therapeutic index in all of medicine. As such, the dose of cancer chemotherapeutic agents generally is limited by undesirable toxicity to the patient being treated. Thus, the ability of an APO4 selective binding agent to target a drug to cancerous prostate tissue allows for a higher amount of chemotherapeutic agent to be delivered directly to the cancer with reduced side effects.

As used herein, the term "therapeutic moiety" means a physical, chemical, or biological material that is linked to an APO4 selective binding agent for the purpose of being targeted *in vivo* to an APO4 polypeptide. A therapeutic moiety can be a cancer chemotherapeutic agent such as doxorubicin, which, when linked to an APO4 selective binding agent, provides a conjugate useful for treating a cancer in a subject. In addition, a therapeutic moiety can be a drug delivery vehicle such as a chambered microdevice, a cell, a liposome or a virus, which can contain an agent such as a drug or a nucleic acid. An APO4 selective binding agent also can be linked

to a therapeutic moiety expressed by a virus, for example, the adenovirus penton base coat protein, thus providing a means to target a virus to cancerous prostate cells (Wickman et al., Gene Ther. 2:750-756 (1995); Weitzman et al., In: "Gene Therapy and Vector Systems" 2:17-25 (1997), each of which is incorporated herein by reference).

A therapeutic moiety also can be, for example, a radioactive moiety or can be a cytotoxic agent, including a toxin such as ricin or a drug such as a chemotherapeutic agent or can be a physical, chemical or biological material such as a liposome, microcapsule, micropump or other chambered microdevice, which can be used, for example, as a drug delivery system. Generally, such microdevices, should be nontoxic and, if desired, biodegradable. Various moieties including microcapsules, which can contain an agent, are well known in the art and commercially available (see, for example, "Remington's Pharmaceutical Sciences" 18th ed. (Mack Publishing Co. 1990), chapters 89-91; Harlow and Lane, Antibodies: A laboratory manual (Cold Spring Harbor Laboratory Press 1988), each of which is incorporated herein by reference).

In one embodiment, an APO4 selective binding agent for treating prostate cancer is a monoclonal or polyclonal antibody. Use of antibody-conjugated immunotoxins and radioactive moieties for the treatment of cancer are well known in the art, as described, for example, by Geissler et al. Cancer Res., 52:2907-2915 (1992); Thrush et al., Ann Rev. Immunol., 14:49-71 (1996); and Trail et al., Science, 261:212-215 (1993). For example, the anthracyclin, doxorubicin, has been linked to antibodies and the antibody/doxorubicin

conjugated have been therapeutically effective in treating tumors (Sivan et al., Cancer Res. 55:2352-2356 (1995); Lau et al., Bioorg. Med. Chem. 3:1299-1304 (1995); Shih et al., Cancer Immunol. Immunother. 38:92-98 (1994), which are incorporated herein by reference). Similarly, other anthracyclins, including idarubicin and daunorubicin, have been chemically conjugated to antibodies, which have delivered effective doses of the therapeutic moiety to tumors (Rowland et al., Cancer Immunol. Immunother. 37:195-202 (1993); Aboud-Pirak et al., Biochem. Pharmacol. 38:641-648 (1989)). Thus, an anthracyclin, such as doxorubicin, idarubicin or daunorubicin, is a therapeutic moiety particularly useful in the methods of the invention.

A therapeutic moiety can be conjugated to a selective binding agent using a variety of methods. A covalent bond can be formed, for example, using glutaraldehyde, a heterobifunctional cross-linker or a homobifunctional cross-linker. Additional conjugation methods include carbodiimide conjugation methods (Bauminger and Wilchek, Meth. Enzymol. 70:151-159 (1980), which is incorporated herein by reference). Carbodiimides comprise a group of compounds that have the general formula R-N=C=N-R', where R and R' can be aliphatic or aromatic, and are used for synthesis of peptide bonds. The preparative procedure is simple, relatively fast, and is carried out under mild conditions. Carbodiimide compounds attack carboxylic groups to change them into reactive sites for free amino groups. Carbodiimide conjugation has been used to conjugate a variety of compounds to carriers for the production of antibodies.

In addition to using carbodiimides for conjugation, the water soluble carbodiimide, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) also can be used for conjugating a therapeutic moiety to an APO4 selective binding agent. EDC can also be used to prepare active esters such as N-hydroxysuccinimide (NHS) ester. The NHS ester, which binds only to amino groups, then can be used to induce the formation of an amide bond with the single amino group of the doxorubicin. The use of EDC and NHS in combination is commonly used for conjugation in order to increase the yield of conjugate formation (Bauminger and Wichek, *supra*, 1980).

Other methods for conjugating a therapeutic moiety to an APO4 selective binding agent also can be used. For example, sodium periodate oxidation followed by reductive alkylation of appropriate reactants can be used, as can glutaraldehyde cross-linking. The cross-linking of proteins can additionally be accomplished by using reactive groups within the individual protein such as carbohydrate, disulfide, carboxyl or amino groups. Coupling can also be accomplished by oxidation or reduction of the native protein, or treatment with an enzyme, for example. However, it is recognized that, regardless of which method of producing a conjugate is selected, a determination must be made that the APO4 selective binding agent maintains its targeting ability and that the therapeutic moiety maintains its relevant function.

The yield of therapeutic moiety/selective binding agent conjugate formed is determined using routine methods. For example, HPLC or capillary electrophoresis or other qualitative or quantitative method can be used (see, for example, Liu et al., J.

<u>Chromatogr.</u> 735:357-366 (1996); Rose et al., <u>J. Chromatogr.</u> 425:419-412 (1988), each of which is incorporated herein by reference). In particular, the skilled artisan will recognize that the choice of a method for determining yield of a conjugation reaction depends, in part, on the physical and chemical characteristics of the specific therapeutic moiety and selective binding agent. Following conjugation, the reaction products are desalted to remove any free peptide and free drug.

A conjugate made up of an APO4 selective binding agent linked to a therapeutic moiety can be administered to an individual having prostate cancer, or, if desired, to an individual suspected of having prostate cancer or an individual at risk of developing prostate cancer. Administration can be accomplished, for example, by intravenous, intraperitoneal or subcutaneous injection. A conjugate made up of an APO4 selective binding agent linked to a therapeutic moiety can be administered by conventional methods using a dosage sufficient to reduce the growth or severity of the prostate cancer. Such dosages can be readily determined by those skilled in the art and include a variety of different regimes, including single high dose administration or repeated small dose administration or a combination of both. The dosing will depend on the progression of the disease and overall health of the individual and can be determined by those skilled in the art.

The present invention also provides a method of treating or reducing the severity of an APO4-mediated disease in a subject by administering an APO4 polypeptide or active fragment thereof or by administering a nucleic acid molecule encoding the APO4 polypeptide or fragment.

An APO4 polypeptide or nucleic acid molecule useful in such a method can be or encode, for example, an amino acid sequence identical or substantially the same as mAPO4-αL (SEQ ID NO:8) or an active fragment thereof, mAPO4-αS (SEQ ID NO:10) or an active fragment thereof, hAPO4-α (SEQ ID NO:12) or an active fragment thereof, rAPO4-α (SEQ ID NO:14) or an active fragment thereof or mAPO4-β (SEQ ID NO:16) or an active fragment thereof, or mAPO4-γ (SEQ ID NO:32) or an active fragment thereof. An APO4 antisense nucleic acid molecule, which has a nucleotide sequence that is identically or substantially complementary to an APO4 encoding nucleic acid or active fragment thereof, also can be used to treat or reduce the severity of an APO4-mediated disease according to a method of the invention.

The present invention also provides a method of treating or reducing the severity of an APO4-mediated disease in a subject such as, for example, prostate cancer, by administering an APO4 regulatory agent to the subject. As used herein, an "APO4 regulatory agent" is an agent that inhibits or enhances a biological activity of an APO4 polypeptide. Such an APO4 regulatory agent can be, for example, an inhibitory agent capable of inhibiting the activity of APO4 or an APO4-mediated activity. For example, in the case of prostate cancer, individuals with this disease can be treated with inhibitors of a JNK pathway to reduce the severity of the disease.

An APO4 regulatory agent can also be an APO4 inhibitory agent such as a dominant negative form of APO4, or an APO4 selective binding agent that inhibits a biological activity of an APO4 polypeptide. Dominant negative forms of APO4 include, for example, soluble

forms of APO4 having an APO4 ligand binding domain such as mAPO4-β, as well as membrane bound forms of APO4 having an APO4 ligand binding domain but lacking a functional cytoplasmic tail such as mAPO4-αS, known as "decoy receptors." One skilled in the art understands that such an APO4 regulatory agent can be an agent that selectively regulates a biological activity of an APO4 polypeptide or, alternatively, can be a non-selective agent that, in addition to regulating a biological activity of an APO4 polypeptide, also regulates the activity of, for example, other TNF family receptors.

As disclosed herein, amino acids 194 to 355 are important for activation of the JNK pathway and apoptosis. Since the mAPO4α-short and mAPO4-γ forms lack the above domains, these and similar isoforms of APO4 can lack the ability to induce the JNK pathway or cell death. However, since these isoforms of APO4 possess a complete or almost complete ligand-binding extracellular domain, they can still compete with the full-length form of APO4 for binding to the ligand, thereby blocking signal transduction mediated by the full-length receptor. As such, mAPO4α-short, mAPO4-γ, their species homologs and isoforms, and natural or synthetic compounds mimicking their ligand-binding ability but lacking the complete cytoplasmic domain or the domain encoding the amino acids 194 to 355 (or homologous domains in other species) can be useful therapeutic tools for the treatment of diseases resulting from the dysregulation of APO4 signal transduction pathway. Such agents also can serve as lead compounds for the development of therapeutic compounds useful in the diagnosis and treatment of diseases resulting from the dysregulation of APO4 function.

The APO6 encoding nucleic acids and polypeptides of the invention can be used to diagnose, treat or reduce the severity of an APO6-mediated disease. For example, the APO6 encoding nucleic acids, polypeptides and active fragments thereof can be used for diagnosis of an APO6-mediated disease or can be used to generate reagents useful for such diagnosis. Diagnosis can be performed, for example, by nucleic acid probe hybridization, or by amplification of APO6 encoding nucleotide sequences and subsequent electrophoretic analysis such as DNA sequencing. Diagnosis also can be performed, for example using antibody or ligand based detection with an APO6 selective binding agent. Detection can be performed *ex vivo*, for example, by removing a cell or tissue sample from an individual at risk of, suspected of having or exhibiting one or more symptoms of an APO6-mediated disease. Altered APO6 expression or activity is indicative of an APO6-mediated disease. As used herein, the term "APO6-mediated disease" means a disease, pathology, or other condition resulting from an abnormality in the expression or activity of an APO6 polypeptide. Included within the meaning of the term "APO6-mediated disease" are disorders of cell loss, disorders of cell accumulation and disorders of cell differentiation.

The invention provides a method of treating or reducing the severity of an APO6-mediated disease in a subject by administering an APO6 polypeptide or active fragment thereof or by administering a nucleic acid molecule encoding the APO6 polypeptide or fragment. An APO6 polypeptide or nucleic acid molecule useful in such a method can be or encode, for example, an amino acid sequence identical or substantially the same as hAPO6 (SEQ ID NO:18), or an active fragment thereof. An APO6

antisense nucleic acid molecule, which has a nucleotide sequence that is identically or substantially complementary to an APO6 encoding nucleic acid or active fragment thereof, also can be used to treat or reduce the severity of an APO6-mediated disease according to a method of the invention.

In addition, the invention provides a method of treating or reducing the severity of an APO6-mediated disease in a subject by administering an APO6 regulatory agent to the subject. An "APO6 regulatory agent," as used herein, is an agent that inhibits or enhances a biological activity of an APO6 polypeptide. Such an APO6 regulatory agent can be, for example, an APO6 inhibitory agent such as a dominant negative form of APO6, an alternatively spliced full-length form of APO6 polypeptide or an APO6 selective binding agent that inhibits a biological activity of an APO6 polypeptide. An APO6 regulatory agent also can be an agent that selectively binds an alternatively spliced full-length form of APO6, thereby blocking or transmitting a signal through full-length APO6. One skilled in the art understands that an APO6 regulatory agent can be an agent that selectively regulates a biological activity of an APO6 polypeptide or, alternatively, can be a non-selective agent that, in addition to regulating a biological activity of an APO6 polypeptide, also regulates the activity of, for example, other TNF family receptors.

The TNRL1 encoding nucleic acids and polypeptides of the invention can be used to diagnose, treat or reduce the severity of a TNRL1-mediated disease. For example, the TNRL1 encoding nucleic acids, polypeptides and active fragments thereof can be used for

diagnosis of a TNRL1-mediated disease or can be used to generate reagents useful for such diagnosis. Diagnosis can be performed, for example, by nucleic acid probe hybridization, or by amplification of TNRL1 encoding nucleotide sequences and subsequent electrophoretic analysis such as DNA sequencing. Diagnosis also can be performed using, for example, antibody or ligand based detection with a TNRL1 selective binding agent. Detection can be performed *ex vivo*, for example, by removing a cell or tissue sample from an individual at risk of, suspected of having or exhibiting one or more symptoms of a TNRL1-mediated disease. Altered TNRL1 expression or activity is indicative of a TNRL1-mediated disease. As used herein, the term "TNRL1-mediated disease" means a disease, pathology, or other condition resulting from an abnormality in the expression or activity of a TNRL1 polypeptide. Included within the meaning of the term "TNRL1-mediated disease" are disorders of cell loss, disorders of cell accumulation and disorders of cell differentiation.

The invention also provides a method of treating or reducing the severity of a TNRL1-mediated disease in a subject by administering a TNRL1 polypeptide or active fragment thereof or by administering a nucleic acid molecule encoding the TNRL1 polypeptide or fragment. A TNRL1 polypeptide or nucleic acid molecule useful in the method can be or encode, for example, an amino acid sequence identical or substantially the same as hTNRL1-α (SEQ ID NO:20) or an active fragment thereof, mTNRL1-α (SEQ ID NO:22) or an active fragment thereof, hTNRL1-β (SEQ ID NO:24) or an active fragment thereof, or mTNRL1-β (SEQ ID NO:26) or an active fragment thereof. A TNRL1 antisense nucleic acid molecule, which has a nucleotide sequence that is identically or substantially

complementary to a TNRL1 encoding nucleic acid or active fragment thereof, also can be used to treat or reduce the severity of a TNRL1-mediated disease.

In addition, the invention provides a method of treating or reducing the severity of a TNRL1-mediated disease in a subject by administering a TNRL1 regulatory agent to the subject. A "TNRL1 regulatory agent," as used herein, is an agent that inhibits or enhances a biological activity of a TNRL1 polypeptide. A TNRL1 regulatory agent can be, for example, a TNRL1 stimulatory agent such as a soluble form of TNRL1 having a TNRL1 receptor binding domain. A TNRL1 regulatory agent also can be, for example, a TNRL1 selective binding agent that inhibits a biological activity of a TNRL1 polypeptide. Such a TNRL1 selective binding agent can be, for example, a small peptide, organic molecule or other agent which competes with TNRL1 for binding to a TNRL1 receptor but which, when bound, does not lead to receptor aggregation or signal transduction. One skilled in the art understands that a TNRL1 regulatory agent can be an agent that selectively regulates a biological activity of a TNRL1 polypeptide or, alternatively, can be a non-selective agent that, in addition to regulating a biological activity of a TNRL1 polypeptide, also regulates the activity of, for example, other TNF family ligands.

The TNRL3 encoding nucleic acids and polypeptides of the invention also can be used to diagnose, treat or reduce the severity of a TNRL3-mediated disease. For example, the TNRL3 encoding nucleic acids, polypeptides and active fragments thereof can be used for diagnosis of a TNRL3-mediated disease or can be used to generate reagents useful for such

diagnosis. Diagnosis can be performed, for example, by nucleic acid probe hybridization, or by amplification of TNRL3 encoding nucleotide sequences and subsequent electrophoretic analysis such as DNA sequencing. Diagnosis also can be performed, for example, using antibody or ligand based detection with a TNRL3 selective binding agent. Detection can be performed *ex vivo*, for example, by removing a cell or tissue sample from an individual at risk of, suspected of having or exhibiting one or more symptoms of a TNRL3-mediated disease. Altered TNRL3 expression or activity is indicative of a TNRL3-mediated disease. As used herein, the term "TNRL3-mediated disease" means a disease, pathology, or other condition resulting from an abnormality in the expression or activity of a TNRL3 polypeptide. Included within the meaning of the term "TNRL3-mediated disease" are disorders of cell loss, disorders of cell accumulation and disorders of cell differentiation.

The invention also provides a method of treating or reducing the severity of a TNRL3-mediated disease in a subject by administering a TNRL3 polypeptide or active fragment thereof or by administering a nucleic acid molecule encoding the TNRL3 polypeptide or fragment. A TNRL3 polypeptide or nucleic acid molecule useful in the method can be or encode, for example, an amino acid sequence identical or substantially the same as hTNRL3 (SEQ ID NO:28) or an active fragment thereof, or mTNRL3 (SEQ ID NO:30) or an active fragment thereof. A TNRL3 antisense nucleic acid molecule, which has a nucleotide sequence that is identically or substantially complementary to a TNRL3 encoding nucleic acid or active fragment thereof, also can be used to treat or reduce the severity of a TNRL3-mediated disease.

In addition, the invention provides a method of treating or reducing the severity of a TNRL3-mediated disease in a subject by administering a TNRL3 regulatory agent to the subject. A "TNRL3 regulatory agent," as used herein, is an agent that inhibits or enhances a biological activity of a TNRL3 polypeptide. A TNRL3 regulatory agent can be, for example, a TNRL3 stimulatory agent such as a soluble form of TNRL3 having a TNRL3 receptor binding domain. A TNRL3 regulatory agent also can be, for example, a TNRL3 selective binding agent that inhibits a biological activity of a TNRL3 polypeptide. Such a TNRL3 selective binding agent can be, for example, a small peptide, organic molecule or other agent which competes with TNRL3 for binding to a TNRL3 receptor but which, when bound, does not lead to receptor aggregation or signal transduction. One skilled in the art understands that such a TNRL3 regulatory agent can be an agent that selectively regulates a biological activity of a TNRL3 polypeptide or, alternatively, can be a non-selective agent that, in addition to regulating a biological activity of a TNRL3 polypeptide, also regulates the activity of, for example, other TNF family ligands.

The present invention also provides a method of treating or reducing the severity of a disorder of cell loss. Examples of such pathologies, which can result from excessive programmed cell death, include AIDS and degenerative disorders such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, retinitis pigmentosa, and cerebellar degeneration. Disorders of cell loss caused by excessive programmed cell death also include myelodysplastic syndromes such as aplastic anemia and ischemic injuries such as myocardial infarction, stroke and reperfusion injury. Any such

pathology is encompassed by the term "disorder of cell loss," which, as used herein, means a disease, pathology or other condition characterized by excessive cell death or differentiation or inadequate cellular proliferation resulting in an abnormally reduced number of cells.

Thus, the invention provides a method of treating or reducing the severity of a disorder of cell loss in a subject by administering an agent that selectively decreases APO8 apoptotic activity, thereby inhibiting apoptosis in the subject. The invention also provides a method of treating or reducing the severity of a disorder of cell loss in a subject by administering an agent that selectively decreases apoptotic activity, where the apoptotic activity is selected from the group selected from APO9 apoptotic activity, APO4 apoptotic activity or APO6 apoptotic activity. Such methods can be used to treat, for example, acquired immunodeficiency syndrome, which is characterized by an increased level of apoptosis in an infected T cell as compared to a normal T cell. The methods of the invention for treating or reducing the severity of a disorder of cell loss in a subject also can be used to treat neurodegenerative disorders such as Alzheimer's disease or Parkinson's disease, the disorders of cell loss described above or another disorder of cell loss known in the art.

As used herein, an agent that "selectively decreases APO8 apoptotic activity" produces a substantially greater reduction in the apoptotic activity of an APO8 polypeptide relative to any reduction in the apoptotic activity of another polypeptide, for example, another pro-apoptotic TNF receptor such as TNFR-1. Similarly, an agent that selectively decreases APO9, APO4 or APO6 apoptotic activity produces a substantially

greater reduction in the apoptotic activity of an APO9 polypeptide, an APO4 polypeptide, or an APO6 polypeptide, respectively, relative to any reduction in the apoptotic activity of an unrelated polypeptide.

The present invention also provides a method of treating or reducing the severity of a disorder of increased cell accumulation. Disorders of increased cell accumulation include cancers such as lymphomas, carcinomas, and hormone dependent tumors such as breast, prostrate and ovarian tumors. Additionally, autoimmune diseases, such as myasthenia gravis, rheumatoid arthritis, systemic lupus erythematosus and immune-mediated glomerulonephritis, and viral infections such as herpesvirus, poxvirus and adenovirus can be a disorder of increased cell accumulation. Pathological conditions involving smooth or cardiac muscle cells, such as hepatic necrosis vasculitis, angiogenesis, atherosclerosis and myocarditis, also can result from increased cell accumulation. Any such pathology is encompassed within the term "disorder of increased cell accumulation," which, as used herein, means a disease, pathology or other condition characterized by diminished cell death or differentiation or excess cell proliferation resulting in an abnormally high number of cells.

Provided herein is a method of treating or reducing the severity of a disorder of cell accumulation in a subject by administering an APO8 polypeptide or active fragment thereof, or a nucleic acid molecule encoding the APO8 polypeptide or active fragment, to the subject. The present invention also provides a method of treating or reducing the severity of a disorder of cell accumulation in a subject by administering an APO9

polypeptide or active fragment thereof, or a nucleic acid molecule encoding the APO9 polypeptide or fragment, to the subject. Further provided herein is a method of treating or reducing the severity of a disorder of cell accumulation in a subject by administering an APO4 polypeptide or active fragment thereof, or a nucleic acid molecule encoding the APO4 polypeptide or fragment, to the subject. The invention also provides a method of treating or reducing the severity of a disorder of cell accumulation in a subject by administering an APO6 polypeptide or active fragment thereof, or a nucleic acid molecule encoding the APO6 polypeptide or fragment, to the subject.

The TNRL1 and TNRL3 polypeptides of the invention also can be useful in treating or reducing the severity of the disorder of cell accumulation. The present invention provides a method of treating or reducing the severity of a disorder of cell accumulation in a subject by administering a TNRL1 polypeptide or active fragment thereof, or a nucleic acid molecule encoding the TNRL1 polypeptide or fragment, to the subject. Also provided herein is a method of treating or reducing the severity of a disorder of cell accumulation in a subject by administering a TNRL3 polypeptide or active fragment thereof, or a nucleic acid molecule encoding the TNRL3 polypeptide or fragment, to the subject.

Further provided herein is a method of treating or reducing the severity of a disorder of cell accumulation in a subject by administering an agent that selectively increases APO8 apoptotic activity, thereby enhancing apoptosis in the subject. The invention also provides a method of treating or reducing the severity of

a disorder of cell accumulation in a subject by administering an agent that selectively increases apoptotic activity, where the apoptotic activity is selected from the group consisting of APO9 apoptotic activity, APO4 apoptotic activity or APO6 apoptotic activity. The present invention further provides a method of treating or reducing the severity of a disorder of cell accumulation in a subject by administering an agent that selectively increases apoptotic activity, where the apoptotic activity is selected from the group consisting of TNRL1 apoptotic activity and TNRL3 apoptotic activity. The methods of the invention for treating or reducing the severity of a disorder of cell accumulation can be used to treat, for example, cancers such as breast, prostrate and ovarian tumors, lymphomas and carcinomas as well as the disorders of cell accumulation described above or another disorder of cell accumulation known in the art.

As used herein, an agent that "selectively increases APO8 apoptotic activity" means an agent that produces a substantially greater enhancement of the apoptotic activity of an APO8 polypeptide relative to any enhancement of the apoptotic activity of another polypeptide, for example, another pro-apoptotic TNF receptor such as CD27. Similarly, an agent that selectively increases APO9, APO4, or APO6 apoptotic activity produces a substantially greater enhancement of the apoptotic activity of an APO9 polypeptide, an APO4 polypeptide, or an APO6 polypeptide, respectively, relative to any enhancement of the apoptotic activity of an unrelated polypeptide. In addition, an agent that selectively increases TNRL1 or TNRL3 apoptotic activity produces a substantially greater enhancement of the apoptotic activity of a TNRL1 polypeptide or TNRL3

polypeptide, respectively, relative to any enhancement of an unrelated polypeptide such as TNF-α.

The following examples are intended to illustrate but not limit the present invention.

EXAMPLE I

Identification and Characterization of human APO8 and APO8 related polypeptide

This example describes the identification and characterization of nucleic acid sequences encoding full-length human APO8 and the APO8 related polypeptide APO8RP.

Isolation and characterization of the hAPO8 cDNA

APO8 is a human cDNA related to IMAGE consortium clones 650744 and 664665, which were derived from a Stratagene NT neuronal library. These clones were identified by searching the EST database (dbEST) for sequences with homology to the extracellular domain of human TNFR-1 protein using the TBLASTN algorithm 5' and 3' RACE was performed on human fetal brain Marathon-ready mRNA (Clontech, Palo Alto, CA) according to the manufacturer's instructions. 3' RACE was performed with primer SEQ ID NO:34 (5'-CTGTTGGATCCAGCTGAGTCTGCTCTGATCACCCAAC-3') and AP1 primer SEQ ID NO:38 (5'-CCATCCTAATACGACTCATATAGGGC-3'). The subsequent round of nested PCR was performed with primer SEQ ID NO:35 (5'-GGACACCATATCTCAGAAGACGGTAGAG-3') AP2 primer SEQ ID NO:36 (5'-ACTCATATAGGGCTCGAGCGGC-3'). 5' RACE was performed with primer SEQ ID NO:37 (5'-TATAGTCCTGTCCATATTTGCAGGAGATG-3') and the AP1 primer SEQ ID NO:38. The subsequent round of nested PCR was

performed with primer SEQ ID NO:38 (5'-CTCTACCGTCTTCTGAGATATGGTGTCC-3') and the AP2 primer SEQ ID NO:36. RACE fragments were cloned into the PCR2.1 vector using the Original TA Cloning kit (Invitrogen, Carlsbad, CA). Inserts from eight or more individual clones were PCR amplified using flanking vector primers Vn26 (SEQ ID NO:39; 5'-TTTCCCAGTCACGACGTTGTA-3') and Vn27 (SEQ ID NO:40; 5'-GTGAGCGGATAACAATTTCAC-3') and purified using the Wizard PCR kit (Promega, Madison, WI). The inserts were sequenced on an ABI 373 automated fluorescent sequencer with dye terminators using purified PCR product or plasmid DNA as template and primers SEQ ID NOS:39 and 40 as the sequencing primers. Percentage identity and similarity scores were obtained using the Gap program (Genetic Computer Group, Madison, WI).

The isolated full-length APO8 cDNA nucleotide sequence, provided as SEQ ID NO:1, encodes a polypeptide of 411 amino acids with characteristics of a cell surface receptor, including an N-terminal signal peptide of 51 amino acids and a transmembrane region of 27 amino acids (residues 185 to 212). The context of the initiating methionine (CCGCC<u>ATG</u>G) conforms to the Kozak consensus sequence, and an in-frame stop codon ten amino acids upstream of this methionine confirmed that it is the true start site. The extracellular domain (residues 52 to 184) of hAPO8 contains the cysteine-rich pseudo-repeats that are a hallmark of the TNF receptor family and exhibits a high degree of amino acid sequence homology to the TRAIL receptor (DR4) (58% identity and 70% similarity; Pan et al., *supra,* 1997, which is incorporated herein by reference). To a lesser extent, the extracellular domain of hAPO8 is homologous to TNFR-1 (27% amino acid identity and 46% similarity), FAS/APO-1 (25% amino acid identity and 45% similarity), DR3 (21%

amino acid identity and 40% similarity) and other members of the TNFR family (see Table 1). Based on this homology, the APO8 polypeptide is classified as a new member of the TNFR family.

hAPO8 has a cytoplasmic tail of 212 amino acids (residues 213 to 411) with a death domain near the C-terminus. As shown in Figure 4B, the APO8 death domain exhibits significant sequence homology to the death domains of DR4, TNFR-1, FAS/APO-1 and DR3. Furthermore, several amino acids that are essential for transmission of the apoptotic signal by TNFR-1 and FAS/APO-1 also are conserved in the death domain of APO8. This includes leucine residue 334, which corresponds to the site of the *lpr* mutation in the murine FAS receptor (see Figure 4B).

Expression of hAPO8 was studied by Northern analysis. A major transcript of about 4.4 kb was seen in all tissues tested, including spleen, thymus, prostate, testis, ovary, small intestine, colonic mucosa and peripheral blood lymphocyte (PBL), with the highest level of expression in PBL. A more slowly migrating minor transcript, which may represent the product of alternative splicing, was present in all tissues assayed. These results demonstrate that RNA encoding the human APO8 polypeptide is widely expressed.

Northern analysis was performed using a multiple human tissue RNA blot (#7759-1) from Clontech. The blot was hybridized with a ^{32}P-labeled APO8 cDNA according to the manufacturer's instructions.

Characterization of the APO8 related polypeptide (APO8RP) cDNA

The APO8RP nucleotide sequence, shown in Figure 3A, encodes a polypeptide of 410 amino acids. Alignment of the amino acid sequences of human APO8 (SEQ ID NO:2) and human APO8RP (SEQ ID NO:4) revealed that hAPO8RP exhibits substantial homology to hAPO8 throughout the entire polypeptide sequence. The hAPO8 and hAPO8RP predicted signal peptides, transmembrane regions and death domains are shown in Figure 4A. In particular, the hAPO8RP signal peptide sequence (residues 1 to 51) is homologous to the hAPO8 signal peptide (residues 1 to 51), indicating that the N-terminal portion of the hAPO8RP signal is Met-Gly-Gln-His. Hydrophobicity plots of the hAPO8 and hAPO8RP sequences support the conclusion that the hAPO8 signal peptide corresponds to residues 1 to 51 shown in Figure 4A and that the APO8RP signal peptide corresponds to residues 1 to 51 shown in Figure 4A. Furthermore, two artificial neural networks (Nielsen et al., <u>Protein Eng.</u> 10:1-6 (1997), which is incorporated by reference herein), trained at recognizing eukaryotic signal peptides and their cleavage sites, identified cleavable signal peptides between amino acids 1 to 51 of APO8 and between amino acids 1 to 51 of hAPO8RP, respectively. The results with hAPO8RP contrast with those of Pan et al., *supra,* 1997, which describe a related protein containing an additional 58 N-terminal residues.

Hydrophobicity plots were generated by using the Pepplot program (Genetic Computer Group). Artificial neural networks analysis for recognizing the signal peptides and their cleavage sites was performed using the

Signal V1.1 World Wide Web Server (http://www.cbs.dtu.dk/service

EXAMPLE II

APO8 and DR4 regulate NF-κB expression and induce FADD-dependent apoptosis

This example demonstrates that APO8 and APO8RP induce cell death through the cytoplasmic protein FADD. In addition, the APO8 and APO8RP polypeptides activate NF-κB expression.

APO8 induces apoptosis

The ability of hAPO8 to induce apoptosis was assayed in the human breast carcinoma cell line MCF-7, the human embryonic kidney cell 293T subclone and the baby hamster kidney BHK cell line. Transient transfection of full-length hAPO8 induced rapid apoptosis in all three cell lines tested. In contrast, expression of an APO8 deletion mutant lacking the C-terminal 192 amino acids ("APO8ΔCP") or an APO8 deletion mutant lacking the C-terminal 134 amino acids, which includes the death domain, ("APO8ΔDD") failed to induce apoptosis. Exemplary results in MCF-7 cells are shown in Figure 5A. These data demonstrate that the C-terminal 134 amino acids of hAPO8 including its death domain are essential for transmitting the apoptotic signal. Furthermore, a mutant construct containing a leucine to asparagine substitution at residue 334 ("APO8-L334N") also failed to induce apoptosis (Figure 4A). Leucine 334 corresponds to the site of the *lpr* mutation and is essential for apoptotic signaling in TNFR-1, FAS/APO-1 and DR3. These results indicate that, like TNFR-1, FAS/APO-1 and DR3, leucine residue 334 is required for apoptotic activity of hAPO8.

APO8 and APO8RP induced apoptosis is mediated by FADD/MORT1

The adaptor molecule FADD/MORT1 mediates apoptosis by the death domain containing receptors TNFR-1, FAS/APO-1 and DR3 either directly or through an intermediate adaptor molecule TRADD. For example, dominant negative FADD can effectively block the apoptosis mediated by these death receptors. A dominant negative FADD construct consisting of the FADD death domain was tested for the ability to modulate APO8 and APO8RP mediated apoptosis in 293T cells. Increasing amounts of dominant negative FADD led to progressive inhibition of apoptosis mediated by DR3, which has previously been shown to mediate cell death through FADD. As shown in Figure 5B, progressive inhibition of apoptosis was also observed for the hAPO8 and hAPO8RP receptors, with almost complete inhibition of apoptosis at a receptor to dominant negative FADD DNA ratio of 1:10. Dominant negative FADD also inhibited APO8 mediated apoptosis in MCF-7 cells (Figure 5C). These results demonstrate that both hAPO8 and hAPO8RP induced apoptosis is dependent upon FADD/MORT. Thus, a variety of death domain containing receptors (TNFR-1, FAS/APO-1, DR3, hAPO8 and hAPO8RP) induce apoptosis through the FADD/MORT adaptor molecule.

Apoptotic signaling by the TNF receptors TNFR-1 and DR3 involves FADD-mediated recruitment of the ICE protease FLICE/MACH1 (Caspase 8) to the aggregated death receptor complex. To test whether hAPO8 induced apoptosis also involves recruitment of FLICE/MACH1, the ability of a dominant negative FLICE mutant to block APO8-induced apoptosis was tested. This FLICE mutant contains a cysteine to serine substitution at the

catalytic site and is designated "FLICE-C360S." As shown in Figure 5C, this FLICE-C360S mutant was able to effectively block apoptosis mediated by APO8. In view of previous results demonstrating that FLICE interacts with FADD, these data provide further evidence that APO8-induced apoptosis relies on a FADD-dependent pathway.

Several caspase inhibitors, z-VAD-fmk and CrmA, have previously been shown to block apoptosis of TNF death receptors. The ability of these caspase inhibitors to effect APO8 mediated apoptosis was assayed, and the results are shown in Figure 5C. These results demonstrate that z-VAD-fmk and CrmA each inhibit APO8-induced apoptosis, indicating that APO8 causes cell death through a caspase-dependent pathway.

Myc and 6X-His epitope tagged APO8 constructs were prepared by amplifying amino acids 51 to 411 of APO8 using *pfu* polymerase (Stratagene, La Jolla, CA) with a 5' primer containing a BamHI site and a 3' primer containing a SalI site. The amplified APO8 fragment was subsequently ligated to a modified pSecTag A vector (Invitrogen) containing a Myc or an MRG-6XHis epitope tag downstream of a murine Ig κ-chain signal peptide. The APO8-L334N construct, which has a leucine to asparagine mutation at residue 334, was generated by mutagenesis of the 6X-His-APO8 plasmid with primers SEQ ID NO:41 (5'-CGCTCATGAGGAAGTTGGGCAACATGGACAATGAGATAAAG-3') and SEQ ID NO:42(5'-CTTTATCTCATTGTCCATGTTGCCCAACTTCCTCATGAGCG-3') using the Quickchange kit (Stratagene) according to the manufacturer's instructions. Deletion construct APO8ΔCP lacks the 192 C-terminal residues of full-length APO8 and was constructed by creating a PCR generated XbaI site in the wild type APO8 sequence. Deletion construct APO8ΔDD

lacks the C-terminal 134 residues of APO8 and was prepared using a naturally occurring SmaI site in the APO8 sequence. The sequences of each of the above constructs were confirmed by automated fluorescent sequencing.

Epitope-tagged versions of APO8RP (amino acids 52 to 410), DR3 (amino acids 27 to 418) and FAS/APO1 (amino acids 19 to 335) were constructed as described above for the APO8 expression vector except that the 5' primer for amplifying Fas/APO1 contained an EcoRI site and the resulting PCR product was cloned into the EcoRI and SalI sites of the modified pSecTag A vector.

The dominant negative FADD construct (DN-FADD) encodes amino acids 80 to 208 of human FADD in pCDNA3 (Invitrogen) and was prepared by Michael Wright of the University of Washington. The FLICE-C360S mutant, which contains a cysteine to serine substitution at residue 360, was prepared with primers SEQ ID NO:43 (5'-GTGTTTTTATTCAGGCTAGTCAGGGGGATAACTACCAGAA-3') and SEQ ID NO:44 (TTCTGGTAGTTATCCCCCTGACTAGCCTGAATAAAAACAC-3') using Stratagene's Quickchange kit. The N-terminal TRADD deletion mutant ND-TRADD-HA, which lacks the N-terminal 102 amino acids, and RIP-HA tagged constructs were prepared using custom primers designed to generate a C-terminal HA-tag. The sequence of each of the above constructs was confirmed by automated fluorescent sequencing.

For transient transfection assays, 0.8-1 X 10^5 MCF-7 or BHK cells were cotransfected with 1 μg of various test plasmids and 200 ng of a *lacZ* expression plasmid in a 24-well plate using 3 μl of Superfect (Qiagen, Santa Clarita, CA) in DMEM supplemented with 10%

FCS essentially according to the manufacturer's instructions. After 3 hr, 0.5 ml of fresh DMEM supplemented with 10% FCS was added. Twenty-four to thirty-six hours later, cells were fixed with 0.05% glutaraldehyde in PBS and stained with 5-bromo-4-chloro-3-indolyl-β-D-galactosidase (X-GAL). For transient transfection of 293T cells, 1 x 10^5 cells were transfected by calcium phosphate precipitation in a 24-well plate essentially as described in Sambrook et al., *supra,* 1989. The data shown in Figure 5A represent the mean +/- the standard deviation determined from three independent experiments performed in duplicate. The percentage of apoptotic cells was calculated as the percentage of cells showing β-galactosidase activity and apoptotic morphology as indicated by a round and condensed shape as compared to the total number of blue staining cells.

For the dominant negative FADD experiments shown in Figure 5B, 150 ng of each receptor (APO8, APO8RP or DR3) was transfected into 293T cells with 150 ng, 750 ng or 1500 ng of DN-FADD DNA. The total amount of DNA in each transfection was held constant by adding control vector plasmid DNA. The results shown in Figure 5B are representative of two independent experiments performed in duplicate.

For the inhibition experiments shown in Figure 5C, MCF-7 cells were transfected with the Myc-APO8 expression construct and a control empty expression vector or an expression vector encoding DN-FADD, FLICE-C360S or CrmA. For experiments with the protease inhibitor z-VAD-fmk, cells transfected with Myc-APO8 and the control vector were treated with 20 μM z-VAD-fmk twelve hours after transfection. Cells were transfected

and scored for apoptotic morphology as described above. The data shown in Figure 5C are representative of three independent experiments performed in duplicate.

APO8 and APO8RP activate NF-κB by a TRADD dependent pathway

Activation of TNFR-1 and DR3 has been shown to induce NF-κB expression, and NF-κB activation can block apoptosis. To test the possibility that APO8 can regulate NF-κB expression, APO8 expression constructs were cotransfected into 293T cells with a NF-κB luciferase reporter containing four copies of an NF-κB binding site. As shown in Figure 5D, overexpression of APO8 produced significant activation of NF-κB in 293T cells, while C-terminal deletion mutants APO8ΔCP and APO8ΔDD and the APO8-L334N point mutant did not activate NF-κB. Similarly, overexpression of APO8RP activated NF-κB as compared to control expression vector (see Figure 5D). These results show that APO8 activates NF-κB expression and that the APO8 death domain is required for this activation. In addition, these results show that APO8RP, in contrast to the DR4 receptor described in Pan et al., *supra,* 1997, also activates NF-κB expression.

NF-κB activation experiments were performed as follows using a NF-κB luciferase reporter with four NF-κB binding sites and a minimal thymidine kinase promoter (Berberich et al., J. Immunol. 153:4357-4366 (1994), which is incorporated herein by reference). 293T cells were cotransfected in duplicate as described above with 0.5 μg of Myc-tagged or 6XHIS-tagged receptor expression construct, 0.5 μg NF-κB reporter construct and 0.2 μg *lacZ* expression construct. After eighteen hours, cells in one well were fixed and stained with X-GAL to

determine the relative transfection efficiency. Cell extracts were prepared from the duplicate well and analyzed for luciferase activity using the Luciferase Assay system (Promega, Madison, WI) following the manufacturer's instructions. Shown in Figure 5D is the mean +/- standard deviation calculated from three independent representative experiments.

Dominant negative TRADD can block NF-κB activation by APO8, APO8RP, and DR3

As described above, the death domain of APO8 is required for APO8-mediated apoptosis and for NF-κB activation. TNFR-1 and DR3 mediated apoptosis and NF-κB activation also are mediated through the TNFR-1 and DR3 death domains, and may be a result of death domain recruitment of TRADD (Kitson et al., Nature 384:372-375 (1996); Tartaglia et al., Cell 74:845-853 (1993), each of which is incorporated herein by reference). TRADD can activate the apoptotic pathway through FADD and can activate the NF-κB pathway through RIP and TRAF2 (Hsu et al., Cell 84:299-308 (1996); Liu et al., Cell 87:565-576, each of which is incorporated herein by reference).

A TRADD mutant deleted for the N-terminal 102 residues (ND-TRADD) lacks a complete TRAF2-binding domain but has a complete RIP/FADD-binding death domain (Liu et al., *supra*, 1996). This TRADD mutant may function as a dominant negative by competing with endogenous full-length TRADD for binding to receptor death domains but failing to effectively recruit TRAF2. The ability of dominant negative TRADD (ND-TRADD) to block NF-κB activation by APO8, APO8RP and DR3 was tested in 293T cells.

Briefly, 293T cells were cotransfected in duplicate with an appropriate receptor and ND-TRADD along with NF-κB (0.5 μg) and lacZ (0.2 μg) reporter constructs. The receptor-to-ND-TRADD ratio was 1:5 (0.15 μg to 0.75 μg). The total amount of plasmid was kept constant by adding vector DNA without insert. Eighteen hours post transfection, luciferase activity was measured from one of the duplicate wells using the luciferase assay reagent (Promega) and following the manufacturer's instructions. The cells in the other well were fixed with glutaraldehyde and stained with X-gal to obtain the relative transfection efficiency. Results (mean +/- SD) are from at least four independent experiments.

The results showed that ND-TRADD effectively blocked NF-κB activation by all three death domain receptors. Weak NF-κB activation was observed with ND-TRADD transfected alone in 293T cells, possibly as a result of the ability of ND-TRADD to recruit RIP and, indirectly, TRAF2 through death domain-death domain interactions (Liu et al., *supra,* 1996). In sum, these results indicate that APO8 and APO8RP resemble DR3 and TNFR-1 in activating NF-κB through a TRADD-dependent pathway.

APO8 and APO8RP interact directly with TRADD and RIP but not with FADD

Coprecipitation experiments were conducted to assay whether APO8 or APO8RP interacts directly with the FADD, TRADD or RIP adaptor molecules. Under conditions which supported the interaction of His epitope tagged FAS/APO1 with FADD, both His tagged APO8 and APO8RP failed to coprecipitate AU1-tagged FADD (AU1-FADD). In contrast, HA-tagged ND-TRADD or RIP were coprecipitated

with APO8 or APO8RP. Furthermore, both APO8 and APO8RP co-immunoprecipitated FADD in the presence of ND-TRADD. Thus, these results show that both APO8 and APO8RP, like DR3 and TNFR-1, directly bind TRADD and RIP and indirectly bind FADD through TRADD. The results with APO8RP are contrary to the results reported in Pan et al., *supra*, 1997, with the DR4 receptor. In sum, FADD appears to be the common mediator of apoptosis by all known death domain-containing receptors.

Coimmunoprecipitation experiments between receptors and various adaptor molecules were performed as follows. 293T cells (2 x 10^6) were plated in a 10 mm plate and cotransfected the following day with 5 μg of various epitope tagged receptor plasmids or control vector lacking receptor sequence; 5 μg of various adaptor plasmids; 2 μg of Crm-A plasmid; and 0.5 μg of a Green Fluorescent Protein (GFP) encoding plasmid (pEGFP-N1, Clontech) by calcium phosphate coprecipitation. The following day, cells were examined under a fluorescent microscope to ensure equal transfection efficiency as determined by the expression of the GFP. Twenty-four to thirty-six hours post-transfection, cells were lysed in 1 ml of lysis buffer containing 1% Triton X-100, 20 mM sodium phosphate (pH 7.4), 150 mM NaCl and 1 EDTA-free protease inhibitor tablet per 10 ml (Boehringer Mannheim, Indianapolis, IN), and the cell lysate cleared by centrifugation.

For immunoprecipitation, pre-cleared cells lysate (600 μl) was incubated with 2 μg of anti-Myc monoclonal 9E10 (American Type Culture Collection, Rockville, MD) for 2 hr at 4°C. The lysate was subsequently incubated for 1 hour with 10μl of Protein A/G ultralink beads (Pierce, Rockford, IL) pre-coated

with 1% BSA to reduce nonspecific binding. Beads were washed twice with lysis buffer, followed by two washes in buffer containing 1% Triton X-100, 20 mM sodium phosphate (pH 7.4) and 500 mM NaCl and once with a buffer containing 20 mM Tris-HCl (pH 7.4). Bound proteins were eluted by adding 70 μl of 0.1 M glycine (pH 3.5) and subsequently neutralized with 10μl of 1 M Tris-HCl (pH 8.0). Samples were separated by SDS-PAGE, transferred to a nitrocellulose membrane and analyzed by Western blot analysis. For immunoprecipitation of MRG-6XHis tagged receptors, a cocktail of three monoclonal antibodies (MRG-4XHis, Penta-His and Tetra-His; Qiagen) was used. For precipitation using Nickel-sepharose, cell lysates were incubated for 1 hour with 20μl of Ni-sepharose beads pre-coated with 1% BSA in a buffer containing 1 % Triton X-100, 20 mM sodium phosphate (pH 7.4), 500 mM NaCl and EDTA-free protease inhibitor cocktail. Beads were washed extensively with the above buffer supplemented with 20 mM imidazole, and the bound protein subsequently eluted and analyzed by SDS-PAGE and Western blot analysis as described above.

NF-κB expression protects against APO8-mediated apoptosis

293T cells were transfected with APO8 expression vector in the presence or absence of an NF-κB expression vector. Thirty-six hours later, cells were fixed and stained and morphology observed. Apoptotic morphology was observed in about 55% of APO8-transfected cells in the absence of NF-κB, while only about 30% of APO8-transfected cells were apoptotic when co-transfected with NF-κB. These results indicate that NF-κB protects against APO8-mediated cell killing.

Cell transfections were performed as described above using 1 x 10^5 293T cells and 150 ng APO8 expression vector, 750 ng NF-κB expression vector or control empty vector, and 200 ng lacZ encoding plasmid in 24 well plates. After thirty-six hours, cells were fixed and stained and the percent apoptotic cells determined as described above.

Myc-APO8RP is expressed on the cell surface and binds TRAIL ligand

The cytotoxic ligand TRAIL has been shown to bind the DR4 receptor based on the ability of a soluble DR4 construct to coimmunoprecipitate TRAIL and to block TRAIL-induced apoptosis in MCF-7 cells. Direct binding of APO8RP to TRAIL was assayed by preparing a stable BHK cell line expressing Myc-epitope tagged APO8RP. Cell-surface expression of APO8RP was confirmed by immunofluorescence staining with an anti-Myc antibody using FACS analysis. Subsequently, the ability of FLAG-epitope tagged TRAIL (FLAG-TRAIL) to bind the APO8RP transformant was tested using immunofluorescence staining with an anti-FLAG antibody. The results demonstrated that FLAG-TRAIL binds Myc-APO8RP. These results demonstrate that APO8RP, like DR4, binds the TRAIL ligand.

Epitope tagged TRAIL protein was prepared as follows. A PCR fragment encoding amino acids 96 to 281 of TRAIL was cloned into a modified pET28(b) vector (Novagen Madison, WI), which has a FLAG epitope downstream of the His epitope, and the sequence confirmed by automated fluorescent sequencing. The His-FLAG-TRAIL protein was purified by Nickel-affinity chromatography according to the manufacturer's instructions.

Stable cell lines were generated by cotransfecting 2 x 10^5 BHK cells with 1.5 μg of a Myc-epitope tagged APO8RP expression vector or control vector lacking the receptor coding sequence and 300 ng of an expression vector encoding human DHFR gene using Superfect (Qiagen) according to the manufacture's instructions. Stable transformants were selected in 1 μM methotrexate (Sigma, St. Louis, MO) in DMEM supplemented with 5% dialyzed fetal calf serum. After 10 to 12 days colonies were pooled to generate a mass culture.

Expression of the Myc-APO8RP construct was analyzed by FACS after indirect immunofluorescence labeling using the anti-Myc epitope antibody mAb 9E10 obtained from American Type Culture Collection or a control monoclonal antibody, followed by R-phycoerythrin (R-PE) labeled goat anti-mouse antibody (Fisher). Binding of FLAG-TRAIL (2 μg) to the Myc-APO8RP expressing stable cell lines was detected similarly using the anti-FLAG M2 antibody (IBI Kodak, New Haven, CT) as the primary antibody and R-PE-labeled goat anti-mouse antibody as the secondary antibody.

EXAMPLE III

Identification and Characterization of APO9

This example describes the identification and characterization of alternatively spliced forms of APO9.

APO9 is a human cDNA related to IMAGE consortium clones 470799, 504745, 110226 and 129137, which are clones with homology to human Fas antigen. The partial amino acid sequence of hAPO9S encodes a signal peptide at the 5' end and a hydrophobic transmembrane region at the 3' end as shown in Figure 6. In addition,

the hAPO9S sequence has a stop codon immediately after the transmembrane region. Thus, the hAPO9S cDNA can represent an alternatively spliced non-functional receptor isoform that competes with the full-length receptor for ligand binding.

In its extracellular domain, the hAPO9S amino acid sequence demonstrates significant homology to the extracellular domains of several members of the TNFR family. The greatest degree of homology is seen with the DR4 receptor, with which it shares 57% amino acid sequence identity and 71% amino acid sequence similarity (see Figure 1 and Table 1). Based on this homology, the APO9S cDNA is classified as a new member of the TNFR family.

EXAMPLE IV

Identification and Characterization of APO4 polypeptides

This example describes the identification and characterization of nucleic acid sequences encoding the short and long isoforms of murine, rat and human APO4-α and murine APO4-β. In addition, the ability of hAPO4 to induce cell death through activation of the JNK pathway and the ability of hAPO4 to activate NF-κB expression is demonstrated.

Characterization of murine APO4-α and mAPO4-γ

mAPO4 are murine cDNAs related to IMAGE consortium EST clones 472300 and 427152, which are mouse embryo-derived sequences with homology to the human DR3 receptor. These clones were identified by searching the EST database (dbEST) for homology to the extracellular domain of human DR3 using the TBLASTN algorithm.

The mAPO4-αS cDNA is represented by IMAGE consortium EST clone 472300 and has an open reading frame of 214 amino acids (Figure 7B), including an amino-terminal signal-peptide; cysteine-rich pseudo-repeats with significant homology to other members of TNFR family (20-24% sequence identity and 35-46% sequence similarity); a highly hydrophobic stretch of amino-acids representing the transmembrane region; and a short cytoplasmic tail.

The mAPO4-γ cDNA is represented by the IMAGE consortium EST clone 427152 and has an open leading frame of 150 amino acids. The amino acid sequence of mAPO4-γ is identical to the mAPO4-α form with the N-terminal 149 amino acids containing the signal peptide and the cysteine-rich pseudo repeats representing the majority of the ligand binding domain. However, mAPO4-γ has a stop codon after amino acid 150 and thus represents a soluble receptor lacking a transmembrane domain.

No polyadenylation signal was observed at the 3' end of mAPO4-αS, indicating that the stop codon present in the 3' end can be the result of alternative splicing. 3' Rapid Amplification of cDNA Ends (RACE) was used on murine spleen marathon-ready cDNA to isolate the full length cDNA. The full length cDNA has an open reading frame of 416 amino acids and a unique cytoplasmic tail with no significant homology to other members of TNF receptor family (Figure 7A). Thus, mAPO4-α is a type I membrane protein, having short and long forms. The long mAPO4-α form can have an important role in the regulation of the immune system, while the short form of the receptor is a decoy receptor which can compete with the long (full-length) form for ligand binding, thereby

modulating signal-transduction through the full-length receptor.

The full-length mAPO4-α (mAPO4-αL) sequence was obtained using 3' RACE and murine spleen Marathon-ready cDNA (Clontech) according to the manufacturer's instructions. Primer SEQ ID NO:45 (5'-CCCACCTCCTCCCTACGAACCACACTG-3') was used with AP1 primer SEQ ID NO:34 in a first round of PCR. Primer SEQ ID NO:46 '-GGCTGCTCGCCCTGCTCATCCTGTGTGT-3') and AP2 primer SEQ ID NO:36 were used in a subsequent round of nested PCR. RACE fragments were cloned into the PCR2.1 vector using the Original TA Cloning kit (Invitrogen). Inserts from eight or more individual clones were PCR amplified using flanking vector primers Vn26 (SEQ ID NO:39; 5'-TTTCCCAGTCACGACGTTGTA-3') and Vn27 (SEQ ID NO:40), and the amplified fragments purified using the Wizard PCR Preps kit (Promega). The inserts were sequenced on an ABI 373 automated fluorescent sequencer with dye terminators using the purified PCR products or plasmid DNA as template with the Vn26 (SEQ ID NO:39) and Vn27 (SEQ ID NO:40) primers as sequencing primers.

mAPO4-αL is a cell surface receptor

To demonstrate that mAPO4-αL is a cell surface receptor, Myc-AU1 tagged mAPO4-αL was expressed in BHK cells. Cell surface expression was confirmed by immunofluorescence labeling with the Myc monoclonal antibody 9E10 (American Type Culture Collection). These results show that mAPO4-αL is a cell surface receptor.

The Myc-AU1 tagged mAPO4-αL construct was prepared by PCR amplifying the protein coding region of the mature APO4-αL polypeptide with a 5' primer containing a BamHI site and encoding the AU1 tag and a 3'

primer containing a XhoI site using *pfu* polymerase (Stratagene). The 5' primer was SEQ ID NO:47 (5'-GGATCCAGACACATACCGCTACATTGCATGTAAAGTGAGTTGCGAAACC-3', and the 3' primer was SEQ ID NO:48 (5'-GAAGCTCGAGGCTGAAAGTATGGAAGTGCTG-3'). The resulting amplified product was digested with BamHI and XhoI and ligated to a BamHI/XhoI digested pSecTag A vector modified to include a Myc tag as described above.

Characterization of human APO4-α

Human APO4-α (hAPO4-α) is encoded by a cDNA sequence related to IMAGE consortium clone 340844, which was identified by searching the EST database (dbEST) for sequence homology to the cytoplasmic tail of full-length mAPO4-α protein using the TBLASTN algorithm. The full-length nucleotide sequence encoding hAPO4-α is shown in Figure 7A. This hAPO4-α nucleotide sequence (Figure 7C) encodes a hAPO4-α polypeptide of 423 amino acids having 68.4% amino acid identity and 79.2% amino acid sequence similarity with mAPO4-α.

The full-length hAPO4-α sequence was obtained using 5' RACE and human fetal spleen Marathon-ready cDNA (Clontech) according to the manufacturer's instructions. Primer SEQ ID NO:49 (5'-TAGCTGGCTTCTCATAGTTAGTGC-3') and AP1 primer SEQ ID NO:34 were used for the first round of PCR. Primer SEQ ID NO:50 (5'-CCTGAGTTGATGCTGATTCTACCA-3') and AP2 primer SEQ ID NO:36 were used for a subsequent round of nested PCR. Inserts from eight or more individual clones were PCR amplified and sequenced essentially as described above.

Characterization of rat APO4-α

Rat APO4-α (mAPO4-α) is another APO4 polypeptide; the rAPO4-α cDNA is related to IMAGE consortium clone 319945 which was identified by searching the EST database (dbEST) for sequences with homology to the cytoplasmic tail of mAPO4-α protein using the TBLASTN algorithm. The partial cDNA sequence, shown in Figure 7D, lacks the 5' region. The partial rAPO4-α nucleotide sequence shares 95% amino acid sequence identity and about 81% nucleotide sequence identity with the cytoplasmic domain of mAPO4-α.

Characterization of murine APO4-β

Murine APO4-β (mAPO4-β) is another APO4 polypeptide; the mAPO4-β cDNA is related to IMAGE consortium clone 835418, derived from a Knowles Solter mouse blastocyst library. The IMAGE consortium clone was identified as described above. The partial cDNA sequence, shown in Figure 8, represents the carboxy-terminal 54 residues of the polypeptide. The encoded polypeptide fragment bears about 77% amino acid sequence identity and about 81% amino acid similarity with mAPO4-α. The mAPO4-β fragment also bears about 74% amino acid identity and about 80% amino acid similarity with rAPO4-α.

APO4 Expression in Adult and Embryonic Tissues

Expression of APO4 in adult human tissues was studied by northern analysis using a multiple tissue northern blot obtained from Clontech (7759-1) following the manufacturer's instructions. The protein coding region of hAPO4 cDNA was radiolabeled with P^{32} and used as a probe. High level APO4 expression was seen in the prostate gland, with very low expression seen in other tissues including spleen, thymus, testis, uterus, small intestine, colon and peripheral blood leukocytes.

The prostate gland consists primarily of fibroblast-like stromal cells and glandular epithelial cells, with the latter being the site of origin of prostatic carcinoma. Reverse-transcriptase/polymerase chain reaction (RT-PCR) was used to test the expression of hAPO4 in a prostate carcinoma derived epithelial cell line, LNCaP (ATCC, Bethesda, MD). Total RNA isolated from LNCaP cells was used for the first strand cDNA preparation using random hexamer primers and Superscript II Reverse Transcriptase (Life Technologies). The RT-PCR procedure was performed following the manufacturer's instruction with forward primer SEQ ID NO:57 (5'-GTCATGTAAAGTGACTTGTG-3') and reverse primer SEQ ID NO:58 (5'-GAGCCGTTGTACTGAATGTCCTG-3'). A specific band corresponding to hAPO4 was detected in RNA from LNCaP cells, indicating that APO4 is expressed in epithelial derived prostate cancer cell lines. Essentially similar results were obtained in another prostate cancer cell line, P65.

APO4 expression during embryonic development was assayed by northern analysis of a multiple tissue

northern blot obtained from Clontech (7763-1), which contained polyA RNA from day 7, 11, 15 and 17 mouse embryos. The protein coding region of mAPO4 cDNA was radiolabeled with P^{32} and used as a probe. A strong signal was detected in samples containing RNA from day 11, 15 and 17 embryos, indicating that APO4 is expressed during early embryonic development.

hAPO4 induces apoptosis

The ability of hAPO4 to induce apoptosis was assayed following transient transfection into the human embryonic kidney 293T cell line. Plasmids encoding various inhibitors were cotransfected to test for their ability to modulate hAPO4 mediated apoptosis in 293T cells.

A mammalian expression construct encoding human APO4 was constructed by amplifying the sequence encoding amino acids 23 to 423 of hAPO4 with a 5' primer containing a Bgl II site and a 3' primer containing a stop codon followed by a Sal I site, as described previously for the APO8 construct. Following digestion of the PCR product with Bgl II and Sal I, the resulting product was inserted into a modified pSecTag A vector as described previously.

Expression of the APO4 polypeptide described above was confirmed by transfecting the above construct into 293T cells using calcium phosphate transfection. After 24 hours, cell lysates were prepared, the proteins resolved by SDS-PAGE, and MYC-tagged hAPO4 detected by western blot analysis using rabbit polyclonal antibody against the MYC epitope tag (Santa Cruz Biotechnology, Inc., Santa Cruz, California) following the

manufacturer's instructions. A single band of approximately 55 kDa was detected in cells transfected with the MYC-APO4 construct.

Constructs encoding various inhibitor plasmids were prepared as follows. A K13-ORF (GenBank U90534) was prepared by PCR amplification of the desired coding sequence from a human genomic DNA sample containing KSHV/HHV8 (human herpesvirus 8) genomic DNA (obtained from Dr. Tim Rose; University of Washington, Seattle, WA). Primers for PCR amplification were based on the published sequence of K13-ORF with additional 5' sequence for subsequent restriction digestion and cloning of the amplified insert. Constructs encoding p35 were prepared by using a baculovirus vector pFastBac HTa (Life Technologies, Inc.) as the template. DN-IκBα or IκBα-ΔN (missing the N-terminal 36 amino acids) and IκBα-S32/36A (Brockman et al., Mol. Cell. Biol., 15: 2809-18, 1995); NFκB driven luciferase reporter construct (Berberch et al., J. Immunol., 153:4357-66, 1994); an active site mutant of full-length rat MEKK1 (MEKK1-D1369A) (Xu et al., Proc. Natl. Acad. Sci. USA, 93:5291-5295) and the JNK binding domain of JIP-1 have been described previously (Dickens et al., Science 277:693-696, 1997). DN-mTRAF2, which encodes a TRAF2 polypeptide lacking the first 87 amino acids, was generated by amplifying the DNA encoding amino acids 88 to 501 of mTRAF2 using the IMAGE consortium EST clone 439083 as a template and incorporating a start site (i.e. methionine residue) at the N-terminus.

Cell transfection was performed using calcium phosphate coprecipitation (Sambrook et al., *supra*, 1989). Briefly, 293T cells (1.2 x 10^5 cells) were cotransfected with 100 ng/well hAPO4 expression plasmid or control

plasmid along with lacZ reporter plasmid and 750 ng/well empty vector or one of various inhibitor plasmids (p35, Caspase 8 C360S, dominant-negative FADD (DN-FADD), MRITα1, K13-ORF, DN-IκBα, JNK binding domain (JBD) of JIP-1, BCLxL or the p65 subunit of NF-κB) and lacZ reporter plasmid and were assayed for hAPO4-induced apoptosis. Thirty-six hours after transfection, cells were fixed and stained with X-gal, and the percentage apoptotic cells determined as described previously.

The results shown in Figure 14A indicate that hAPO4 induces cell death and that the hAPO4-induced apoptosis is activated independently of the FADD-Caspase pathway. In particular, several inhibitors of the FADD-Caspase pathway (caspase 8 C360S, DN-FADD, p35, K13-ORF and MRITα1), when cotransfected with hAPO4, failed to block APO4-mediated cell death. These results demonstrate that, unlike a variety of receptors of the TNF family, APO4 can mediate apoptosis independently of the FADD-caspase pathway.

hAPO4 activates the Jun N-terminal kinase (JNK) pathway

Activation of the JNK pathway by hAPO4 was measured using the PathDetect c-Jun Trans-Reporting system (Stratagene, La Jolla, CA). Briefly, 1.2 x 10^5 293T EBNA cells were cotransfected in duplicate with 250 ng/well of hAPO4 expression vector and 750 ng/well of empty vector or inhibitor plasmid (JBD of JIP-1, MEKK1-D1269A, or DN-TRAF2). A fusion-transactivator plasmid containing the GAL4 DNA-binding domain fused to the c-Jun transcription factor (pFA-cJun, 50 ng), a reporter plasmid encoding the luciferase gene downstream of the GAL4 Upstream Activating sequence (pFR-luc,

500 ng) and a β-galactosidase (pRSV/LacZ) reporter construct (75 ng) were also included in the cotransfection. Forty hours after transfection, cell extracts were prepared and luciferase and β-galactosidase activities measured. The luciferase activity was normalized relative to the β-galactosidase activity to control for differences in transfection efficiency.

As shown in Figure 14B, expression of hAPO4 produced significant activation of c-jun. Furthermore, cotransfection of hAPO4 with JBD-JIP, and to a lesser extent with MEKK1-D1369A or DN-TRAF2, prevented APO4-mediated activation of the JNK pathway. These results indicate that APO4-indcued apoptosis can be mediated through activation of the JNK pathway and MEKK1.

hAPO4 induces NF-κB

To test the possibility that APO4 can regulate NF-κB expression, hAPO4 expression constructs were cotransfected into 293T cells with a NF-κB luciferase reporter. Transfection and NF-κB activation experiments were performed as described previously. Inhibitor plasmids (DN-TRAF2 or IκBα-S32/36A) were also cotransfected to determine whether inhibition of the TRAF2 or JNK pathway affects NF-κB activation by hAPO4. As shown in Figure 14C, hAPO4 induced NF-κB expression, and this activation was not blocked by dominant-negative TRAF2. Also as shown in Figure 14C, hAPO4 failed to activate NF-κB in the presence of IκB-S32/36A, an IκB mutant that resists degradation in response to activators of NF-κB. These results demonstrate that APO4 can function, at least in part, through activation of NF-κB. Given the multiple roles of NF-κB and the high level of APO4 expression in embryonic development, these results

indicate that APO4 can stimulate growth in certain contexts, in addition to its pro-apoptotic role described above.

Deletion analysis of APO4

To determine the domains of APO4 which are responsible for JNK activation and apoptosis, deletion analysis was carried out using the following constructs: pSecTag-MYC-APO4 encoding amino acid 23 to 423; pCDNA3.1 HisA-APO4.CP encoding the cytoplasmic domain of APO4 (amino acids 194 to 423); pSecTag-MYC-APO4-C377 encoding amino acids 23 to 377; pSecTag-MYC-APO4-C355 encoding amino acids 23 to 355; pSecTag-MYC-APO4-C286 encoding amino acids 23 to 286; pSecTag-MYC-APO4-C172 encoding the extracellular domain (amino acids 23 to 172).

These constructs were prepared by PCR amplification of the nucleic acid fragment encoding the designated amino acids using custom primers with appropriate restriction enzyme containing sites and by using human APO4 cDNA as template. The desired PCR fragment was subsequently cloned into the modified pSecTag vector described previously or the pCDNA3.1 HisA vector (Invitrogen).

The constructs were subsequently tested for their ability to activate the JNK pathway in 293EBNA cells as described above. The results indicated that, while the full-length APO4 and its cytoplasmic domain were able to activate the JNK pthway, the construct encoding the extracellular domain failed to do so. Furthermore, the pSecTag MYC-APO4-C286 failed to significantly activate the JNK pathway although the

pSecTag MYC-APO4-C377 and C355 constructs could do so. could.

The above constructs were also tested for their ability to induce cell death in the 293T cells using the assay decribed before. While the full-length APO4 polypeptide, its cytoplasmic domain, C377, and C355 constructs were able to effectively induce cell death, the C286 and the C172 constructs were inactive. These results indicate that the C-terminal 68 amino acids are not essential for the APO4-induced activation of the JNK pathway or apoptosis and that these activities reside among the amino acids 194 to 355.

EXAMPLE V

Identification and Characterization of APO6

This example describes the identification and characterization of a nucleic acid sequence encoding human APO6.

Human APO6 (hAPO6) is a cDNA related to IMAGE consortium clones 366305, 592256, 343204 and 591875, which have some similarity with the extracellular domain of human TNFR-2. These clones were identified by searching the EST database (dbEST) for sequences with homology to the extracellular domain of human TNFR-2 protein using the TBLASTN algorithm. The partial nucleotide sequence, lacking both 5' and 3' sequence, is shown in Figure 9. The partial hAPO6 sequence shows significant homology to the extracellular domains of TNFR-2, TNFR-1, and other members of the TNFR family (about 16-33% amino acid sequence identity and about 30-50% amino acid sequence similarity) (Figure 1 and Table

1). Based on this homology, hAPO6 is classified as a new member of the TNFR family.

EXAMPLE VI

Identification and Characterization of Tumor Necrosis Factor Related Ligands

This example describes the identification and characterization of nucleic acid sequences encoding human and murine TNRL1-α and TNRL1-β. This example also demonstrates that human TNRL1-α is a cytotoxic ligand.

***Characterization of human TNRL1*-α**

Human tumor necrosis factor related ligand 1-α (hTNRL1-α) is encoded by a cDNA clone related to IMAGE consortium clones 593690, 115371 and 129696, which are clones with homology to human TNF-α. The full length cDNA encodes a predicted protein of 285 amino acids with significant sequence homology to TNF-α as well as other members of the TNF family (Figures 10 and 11A).

The 5' end of the human TNRL-α sequence was obtained using 5' RACE on Marathon Ready cDNA derived from human leukocytes (Clontech) as described previously. The first round of RACE was performed using AP1 primer SEQ ID NO:34 as the forward primer and SEQ ID NO:59 (5'-GTTGGTGTTTCACTGTCTGCAATCAG-3') as the reverse primer. The race products were diluted 1:50 in water and used for a second round of RACE using forward primer SEQ ID NO:36 and SEQ ID NO:60 (5'-GTTCTGACTGGAGTTGCCTTCTCCTG-3') reverse primer. As in other members of the TNRL family, the 5' sequence encodes a membrane-anchoring domain. This factor, hTNRL1-α, is designated a new member of the

TNF family which can play an important role in the modulation of the immune and nervous systems.

Human TNRL1-α is a cytotoxic ligand

His-FLAG epitope-tagged extracellular portions of hTNRL1-α, hTNRL3 or cytotoxic TRAIL ligand were produced in bacteria. Western analysis of bacterial lysates with an anti-FLAG antibody demonstrated that hTNRL1-α, hTNRL3 and TRAIL extracellular polypeptide portions were produced. BJAB cells were treated with ligand, and cell survival measured after twenty-four hours using the MTT assay in wells pre-coated with anti-FLAG monoclonal antibody. The relative survival of BJAB cells treated with hTNRL1-α, hTNRL3 or TRAIL was significantly reduced as compared to control cells. These results indicate that hTNRL1-α, like TRAIL, is a cytotoxic ligand.

Epitope tagged hTNRL1-α, hTNRL3 and TRAIL polypeptide fragments were prepared in a modified pET28(b) vector (Novagen Madison, WI), which has a FLAG epitope downstream of the His epitope, and the sequences confirmed by automated fluorescent sequencing. The His-FLAG-tagged polypeptide fragments were expressed in bacteria and purified by nickel-affinity chromatography (Qiagen) according to the manufacturer's instructions.

For cell survival assays, a 96-well plate was pre-treated by incubation with 100 µl/well 10 µg/µl anti-FLAG monoclonal antibody for two hours at 37°C. BJAB cells were plated at 2 x 10^4 cells/well and treated with 5 µl purified ligand. Cell survival was measured after 24 hours using the MTT assay (SIGMA) according to the manufacturer's instructions.

Characterization of murine TNRL1-α

Murine tumor necrosis factor related ligand 1-α (mTNRL1-α) is encoded by a cDNA related to IMAGE consortium clones 722549, 803594 and 791670. These clones were identified by searching the dbEST database (NCBI) for sequences with homology to hTNRL1-α polypeptide (SEQ ID NO:20) using the TBLASTN algorithm. Murine TNRL1-α sequence was obtained using 5' RACE on murine spleen Marathon-ready cDNA (Clontech) according to the manufacturer's instructions. Primer SEQ ID NO:51 (5'-GTCTCCGTTGCGTGAAATCTGTGC-3') and AP1 primer SEQ ID NO:34 were used for the first round of PCR. Primer SEQ ID NO:52 (5'-AGGAATTGTTGGGCAGTGTTTTGG-3') and AP2 primer SEQ ID NO:36 were used for a subsequent round of nested PCR. The RACE products were cloned into vector PCR2.1 using the TA cloning kit (Invitrogen) and sequenced on an automated fluorescent sequencer (ABI 373) essentially as described before.

The available mTNRL1-α sequence is shown in Figure 11B. The predicted mTNRL1-α sequence is highly homologous to hTNRL1-α at both the nucleotide and protein levels and indicates a type II membrane protein with a putative N-terminal cytoplasmic domain (amino acids 1-46, a transmembrane domain (amino acids 47-74) and a large extracellular receptor binding domain (amino acids 75-290).

Characterization of human TNRL1-β

Human tumor necrosis factor related ligand 1-β (hTNRL1-β) is encoded by a cDNA related to IMAGE consortium clones 306307, 489038 and 306064, identified as described above, and which exhibit homology to TNRL1-α. The hTNRL1-β sequence, shown in Figure 12A, encodes a predicted polypeptide of 250 amino acids with sequence homology to TNRL1-α and TNF-α as well as other members of TNF family (see Figure 10). The sequence indicates a type II membrane protein with a putative N-terminal cytoplasmic domain (amino acids 1-25), a transmembrane hydrophobic domain (26-50) and a large extracellular receptor binding domain (amino acids 51-250).

Characterization of murine TNRL1-β

Murine tumor necrosis factor related ligand 1-β (mTNRL1-β) is encoded by a cDNA related to IMAGE consortium clone 876879. This clone was identified by searching the dbEST database (NCBI) for sequences with homology to hTNRL-β polypeptide (SEQ ID NO:24) using the TBLASTN algorithm. The full-length mTNRL1-β cDNA sequence is shown in Figure 12B. The sequence indicates a type II membrane protein with a putative N-terminal cytoplasmic domain (amino acids 1-16), a transmembrane hydrophobic domain (amino acids 17-42) and a large extracellular receptor binding domain (amino acids 43-241).

EXAMPLE VII

Identification and Characterization of TNRL3

This example describes the identification and characterization of nucleic acid sequences encoding human and murine TNRL3. This example also demonstrates that human TNRL3 is a cytotoxic ligand.

Characterization of human TNRL3

Human tumor necrosis factor related ligand 3 (hTNRL3) is related to IMAGE consortium clones 154742 and 271670 and Genbank clone C00994. These sequences were identified by searching the dbEST database (NCBI) for sequences with homology to human TRAIL ligand protein using the TBLASTN olgorithym. 5' RACE with human fetal spleen marathon ready cDNA (Clontech) was performed according to the manufacturer's instructions to obtain additional 5' sequence. Primer SEQ ID NO:53 (5'-CGAAGTAGGTGAGGAAGGGGGCAG-3') and AP1 primer SEQ ID NO:34 were used for the first round of PCR. Primer SEQ ID NO:54 (5'-GTAGACAGCCTTCCCCTCATCAAA-3') and AP2 primer SEQ ID NO:36 were used for a subsequent round of nested PCR.

The partial hTNRL3 sequence shown in Figure 13A lacks sequence at the 5' end of the molecule and encodes the ligand-binding extracellular domain. The missing 5' sequence can encode a membrane anchoring sequence. The partial sequence shown in Figure 13A exhibits homology to TRAIL (TNF Related Apoptosis Inducing Ligand) and other members of the TNF family (see Figure 10).

Human TNRL3 is a cytotoxic ligand

BJAB cells were treated with epitope-tagged extracellular portions of hTNRL1-α, hTNRL3 or TRAIL, and cell survival measured after twenty-four hours using the MTT assay as described above. The relative survival of BJAB cells treated with epitope-tagged hTNRL1-α, hTNRL3 or TRAIL extracellular fragments was significantly reduced as compared to control cells. These results indicate that hTNRL3 functions as a cytotoxic ligand.

Characterization of murine TNRL3

Murine tumor necrosis factor related ligand 3 (TNRL3) is encoded by a cDNA related to IMAGE consortium clone 696209. This clone was identified by searching the dbEST database for sequences with homology to hTNRL3 protein (SEQ ID NO:28) using the TBLASTN algorithm. The available sequence of clone 696209 is incomplete at the 5' end. Additional murine TNRL3 sequence was obtained using 5' RACE on murine spleen Marathon-ready cDNA (Clontech) according to the manufacturer's instructions. Primer SEQ ID NO:55 (5'-CACACCGTTCACCAGCAAGTCCAG-3') and AP1 primer SEQ ID NO:34 were used for the first round of PCR. Primer SEQ ID NO:56 (5'-GGTAGACAGCCTTTCCCTCATCAA-3') and AP2 primer SEQ ID NO:36 were used for a subsequent round of nested PCR. The RACE products were cloned into vector PCR2.1 using the TA cloning kit (Invitrogen) and sequenced on an automated fluorescent sequencer (ABI 373).

The partial mTNRL3 sequence, lacking the 5' end, is shown in Figure 13B. The mTNRL3 cDNA encodes a polypeptide with significant sequence homology to hTNRL3.

All journal article, reference, and patent citations provided above, in parentheses or otherwise, whether previously stated or not, are incorporated herein by reference.

Although the invention has been described with reference to the disclosed embodiments, those skilled in the art will readily appreciate that the specific experiments detailed are only illustrative of the invention. It should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

What is claimed is:

1. An isolated APO4 polypeptide, comprising substantially the same amino acid sequence as an APO4 polypeptide, or an active fragment thereof.

2. The isolated polypeptide of claim 1, comprising substantially the same amino acid sequence as an amino acid sequence selected from the group consisting of mAPO4-αL (SEQ ID NO:8) or an active fragment thereof, mAPO4-αS (SEQ ID NO:10) or an active fragment thereof, hAPO4-α (SEQ ID NO:12) or an active fragment thereof, and rAPO4-α (SEQ ID NO:14) or an active fragment thereof.

3. The isolated polypeptide of claim 1, comprising substantially the same amino acid sequence as mAPO4-β (SEQ ID NO:16).

4. The isolated polypeptide of claim 1, wherein said active fragment comprises a soluble form of an APO4 polypeptide having an APO4 polypeptide ligand binding domain.

5. An isolated nucleic acid molecule, comprising a nucleotide sequence encoding substantially the APO4 polypeptide of claim 1, or an active segment thereof.

6. An APO4 selective binding agent, said agent comprising the activity of selectively binding to the APO4 polypeptide of claim 1.

7. A method of diagnosing prostate cancer in an individual, comprising determining the level of APO4 in said individual.

8. A method of treating prostate cancer, comprising administering to an individual having prostate cancer a conjugate comprising an APO4 selective binding agent linked to a therapeutic moiety.

9. A method of identifying an effective pharmacological agent useful in the diagnosis or treatment of a disease associated with APO4 activity, comprising the steps of:

a) contacting an APO4 polypeptide or active fragment thereof with an agent, and

b) determining selective binding of said agent to said APO4 polypeptide or active fragment thereof,

wherein said selective binding indicates that said agent is an effective pharmacological agent useful in the diagnosis or treatment of a disease associated with APO4 activity.

10. The method of claim 9, wherein said APO4 polypeptide or active fragment thereof is an APO4 extracellular ligand binding domain.

11. The method of claim 9, wherein said APO4 polypeptide or active fragment thereof is expressed in a cell or on the surface of a cell.

12. The method of claim 9, wherein said APO4 polypeptide or active fragment thereof is contacted with an agent *in vitro*.

13. A method of screening for an APO4 agonist useful in treating prostate cancer, comprising the steps of:

a) contacting a cell expressing an APO4 polypeptide or active fragment thereof with an agent;

b) assaying for increased APO4 activity,

wherein increased APO4 activity indicates that said agent is an APO4 agonist useful in treating prostate cancer.

14. A method of screening for an APO4 antagonist, comprising the steps of:

a) contacting a cell expressing an APO4 polypeptide or active fragment thereof with an agent;

b) assaying for decreased APO4 activity,

wherein decreased APO4 activity indicates that said agent is an APO4 antagonist.

15. A method of identifying an effective pharmacological agent useful in the diagnosis or treatment of a disease associated with APO4 activity, comprising the steps of:

a) forming a mixture comprising an APO4 polypeptide or active fragment thereof, an APO4 signal transducer molecule that specifically interacts with a cytoplasmic domain of said APO4 polypeptide or active fragment thereof, and an agent;

b) detecting the level of APO4 activity in the presence of said agent,

wherein an alteration in said APO4 activity relative to control activity indicates that said agent is an effective pharmacological agent useful in the diagnosis or treatment of a disease associated with APO4 activity.

16. A method of claim 15, wherein said APO4 activity is measured *in vivo*.

17. A method of claim 15, wherein said APO4 activity is measured *in vitro*.

18. An isolated APO8 polypeptide, comprising substantially the same amino acid sequence as APO8, or an active fragment thereof.

19. The isolated polypeptide of claim 18, comprising substantially the same amino acid sequence as hAPO8 (SEQ ID NO:2), or an active fragment thereof.

20. The isolated polypeptide of claim 18, wherein said active fragment comprises a soluble form of APO8 having an APO8 ligand binding domain.

21. An isolated nucleic acid molecule, comprising a nucleotide sequence encoding substantially the APO8 polypeptide of claim 18, or an active segment thereof.

22. An APO8 selective binding agent, said agent comprising the activity of selectively binding to the APO8 polypeptide of claim 18.

23. An isolated APO9 polypeptide, comprising substantially the same amino acid sequence as APO9, or an active fragment thereof.

24. The isolated polypeptide of claim 23, comprising substantially the same amino acid sequence as hAPO9 (SEQ ID NO:6), or an active fragment thereof.

25. The isolated polypeptide of claim 23, wherein said active fragment comprises a soluble form of APO9 having an APO9 ligand binding domain.

26. An isolated nucleic acid molecule, comprising a nucleotide sequence encoding substantially the APO9 polypeptide of claim 23, or an active segment thereof.

27. An APO9 selective binding agent, said agent comprising the activity of selectively binding to the APO9 polypeptide of claim 23.

28. An isolated APO6 polypeptide, comprising substantially the same amino acid sequence as APO6, or an active fragment thereof.

29. The isolated APO6 polypeptide of claim 28, comprising substantially the same amino acid sequence as hAPO6 (SEQ ID NO:18), or an active fragment thereof.

30. The isolated polypeptide of claim 28, wherein said active fragment comprises a soluble form of APO6 having an APO6 ligand binding domain.

31. An isolated nucleic acid molecule, comprising a nucleotide sequence encoding substantially the APO6 polypeptide of claim 28, or an active segment thereof.

32. An APO6 selective binding agent, said agent comprising the activity of selectively binding to the APO6 polypeptide of claim 28.

33. An isolated tumor necrosis factor related ligand 1 (TNRL1) polypeptide, comprising substantially the same amino acid sequence as TNRL1, or an active fragment thereof.

34. The isolated TNRL1 polypeptide of claim 33, comprising substantially the same amino acid sequence as an amino acid sequence selected from the group consisting of hTNRL1-α (SEQ ID NO:20), or an active fragment thereof, and mTNRL1-α (SEQ ID NO:22), or an active fragment thereof.

35. The isolated TNRL1 polypeptide of claim 33, comprising substantially the same amino acid sequence as an amino acid sequence selected from the group consisting of hTNRL1-β (SEQ ID NO:24), or an active fragment thereof, and mTNRL1-β (SEQ ID NO:26), or an active fragment thereof.

36. The isolated polypeptide of claim 33, wherein said active fragment comprises a soluble form of TNRL1 having a TNRL1 receptor binding domain.

37. An isolated nucleic acid molecule, comprising a nucleotide sequence encoding substantially the TNRL1 polypeptide of claim 33, or an active segment thereof.

38. A TNRL1 selective binding agent, said agent comprising the activity of selectively binding to the TNRL1 polypeptide of claim 33.

39. An isolated TNRL3 polypeptide, comprising substantially the same amino acid sequence as TNRL3, or an active fragment thereof.

40. The isolated TNRL3 polypeptide of claim 39, comprising substantially the same amino acid sequence as an amino acid sequence selected from the group consisting of hTNRL3 (SEQ ID NO:28), or an active fragment thereof, and mTNRL3 (SEQ ID NO:30), or an active fragment thereof.

41. The isolated polypeptide of claim 39, wherein said active fragment comprises a soluble form of TNRL3 having a TNRL3 receptor binding domain.

42. An isolated nucleic acid molecule, comprising a nucleotide sequence encoding substantially the TNRL3 polypeptide of claim 39, or an active segment thereof.

43. A TNRL3 selective binding agent, said agent comprising the activity of selectively binding to the TNRL3 polypeptide of claim 39.

```
         1                                                                                          80
hAPO4-α  .......... .......... .......... .......... ..malkVLLe qektfftlLV LlgyLsckVT cesgdcrqqe
hAPO6    .......... .......... .......... .......... .......... .......... .......... ..........
hAPO8    MeQrGqnApA asGarkrhgP GPReARgArp GLRVPKTLvL VV..aAVLLl VsAeSAlitq qdLapqQrVA PqQkRsSpse
APO8RP   MgQhGpsArA raG....raP GPRpAReAsp rLRVhKTFkF VV..vgVLLq VvpsSAAtik Lh...dQsIg tqQwehSpLg
hAPO9    .......... .......... .......... maRIPKTLkF VVVivAVLLp VlAYSAttar qeevpqQtVA PqQqRhSFkg
CD40     .......... .......... .......... .......... .......... .mvRlplqcV LwgcLltaVh PepPtacrek
Fas      .......... .......... ........ml GiWtllpLvL tsVarlssks VnAqvtdins kgLeLRktVt tvetqn..Le
DR3      .......... .......... .......... .......... ...meqrprG caAvaAAlLl vlLgaRaggg trsPRcdCag
TNFR1    .......... .......... .........m GLstvpdLlL plVllelLvG IypsgvigLV phLgdRekrd svcPqgkY..
TNFR2    .......... .......... .......... .......Map VaVwaAlavG lelWaAAhal paqvaftpyA PepgstcrLr

         81                                                                                         160
hAPO4-α  frdrsgncvp CnqCgPGmeL skECgfgygE DaqCvaCrlh rFkeDWgFqk .....CkpCl dCavvnRfqk ancsaTsdal
hAPO6    .......... .......... .......... .......... .......... ....ercRYc nvlcgeReeE araChaThNr
hAPO8    gL........ ...CPPGhHi ........SE Dgr..dCisC kYGqDYsthw ndLlfCLRCT rCDSg..evE lSpCTTTRNT
APO8RP   EL........ ...CPPGsHr ........SE rpG..aCnrC TeGvgYTnas nnLfaCLpCT aCkSd..eeE rSpCTTTRNT
hAPO9    Ee........ ...CPaGsHr ........SE hTG..aCnpC TeGvDYTnas nnepsCFpCT vCkSd..gkh kSsCTmTRdT
CD40     qYlinsq..C CSlCqPGqkL vsDC..TEft ETeClpCgEs eFldtWnr.. ethchqhkYc dpnlgLRvqq ..kgTsetdT
Fas      gLH.hdgQfC hkpCPPGerk arDC..Tvng Dep..dCvpC qeGkeYTdka hfsSkCrRCr lCDeghglev einCTrTqNT
DR3      DFHkkiglfC CrgCPaGhyL KapC..TEpc gns..tClvC pqdtflaWen hhnSeCaRCq aCDeqasqva lenCsavadT
TNFR1    .iHpQnnsiC CtKChkGtyL ynDC..pgpg qdt..dCrEC esGsftasen hl.rhCLsCs kCrkeMgqvE iSsCTvdRdT
TNFR2    EYydQtaQmC CSKCsPGqHa KvfC..Tkts DTvCdsCeDs TYtqlWnWvp ecLScgsRCs sdqve...tq ..aCTreqNr
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FIG. 1A

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         161                                                                                      240
hAPO4-α  cgdClPGFYR ktklvgfqdm eCvpCgdpPP pYe..phCas kvnlvkIast assprdtalA AvlCsa.... ..........
hAPO6    aCrCrtGFFa h.......ag fCLehasCPP GagVIapgTP sqntqCqPsp pGtFSassSs sEqCqPhrNC TalgLalnvp
hAPO8    VCQCeeGtFR e.edSpEMCR K..CrtgCPr GmvkVgdCTP wSDIeCVhkE sG........ .......... ..........
APO8RP   aCQCKPGtFR n.dnSaEMCR K..CstgCPr GmvkVkdCTP wSDIeCVhkE sGnghN.... .......... ..........
hAPO9    VCQCKeGtFR n.enSpEMCR K..CsR.CPs GevqVsnCTs wdDIqCVeef GanatvetpA AEetmntSpg TpapaaeEtm
CD40     ICtCeeGWhc t...SEa.Ce sCvlhRsCsP GFgVkqiaTg vSDtiCePcp vGfFSNvsSA fEkChPwtsC etkdLvvqqa
Fas      kCrCKPnFFq nstvcE.... ...hCdpCtk cehgIikect ltsntkckeE Gsrs...... .......... ..........
DR3      rCgCKPGWFv ecqvSqcvss spFYCqpCld cg.alhrhTr llcsrrdtdc GtcLpgfyeh gDgCVscpts Tlgscperca
TNFR1    VCgCrkngYR hy......ws enLF..qCfn cslclngtvh lScqekqntv ctchagfflr eneCVscSNC kks...lEct
TNFR2    ICtCrPGWYc alskqEg.CR lCaplRkCrP GFgVarpgTe tSDVvCkPca pGtFSNttSs tDiCrPhqiC n....vvalp

         241                                                                   309
hAPO4-α  .......... .......... .......... .......... .......... .......... .........
hAPO6    GssShDtlCT sctgfPlStr vPGAeecEra Vidfvafqdi sikrLSgccS prgpegWGPt PrAaarpss
hAPO8    .......... .......... .......... .......... .......... .......... .........
APO8RP   .......... .......... .......... .......... .......... .......... .........
hAPO9    nTspgtpapa aeETmttSpg tPapaaeEtm ttspgtpapa aeEtMtTsPg TpasshYlsc t........
CD40     GTnktDvVCg pqD..rl... .......... .......... .......... .......... .........
Fas      .......... .......... .......... .......... .......... .......... .........
DR3      avcgWrQmfw v......... .......... .......... .......... .......... .........
TNFR1    klc.lpQIen vkgTedsg.. .......... .......... .......... .......... .........
TNFR2    GnaSRDaVCT sts..Ptrsm aPGAvhlpqp Vstrsqhtqp tpEp.STaPS TsfllpmGPs PpAegstg.
```

FIG. 1B

```
   1 CGGAGAACCCCGCAATCTCTGCGCCCACAAAATACACCGACGATGCCCGATCTACTTTAA   60
  61 GGGCTGAAACCCACGGGCCTGAGAGACTATAAGAGCGTTCCCTACCGCCATGGAACAACG  120
                                                      M  E  Q  R    4
 121 GGGACAGAACGCCCCGGCCGCTTCGGGGGCCCGGAAAAGGCACGGCCCAGGACCCAGGGA  180
   5  G  Q  N  A  P  A  A  S  G  A  R  K  R  H  G  P  G  P  R  E   24
 181 GGCGCGGGGAGCCAGGCCTGGGCTCCGGGTCCCCAAGACCCTTGTGCTCGTTGTCGCCGC  240
  25  A  R  G  A  R  P  G  L  R  V  P  K  T  L  V  L  V  V  A  A   44
 241 GGTCCTGCTGTTGGTCTCAGCTGAGTCTGCTCTGATCACCCAACAAGACCTAGCTCCCCA  300
  45  V  L  L  L  V  S  A  E  S  A  L  I  T  Q  Q  D  L  A  P  Q   64
 301 GCAGAGAGTGGCCCCACAACAAAAGAGGTCCAGCCCCTCAGAGGGATTGTGTCCACCTGG  360
  65  Q  R  V  A  P  Q  Q  K  R  S  S  P  S  E  G  L  C  P  P  G   84
 361 ACACCATATCTCAGAAGACGGTAGAGATTGCATCTCCTGCAAATATGGACAGGACTATAG  420
  85  H  H  I  S  E  D  G  R  D  C  I  S  C  K  Y  G  Q  D  Y  S  104
 421 CACTCACTGGAATGACCTCCTTTTCTGCTTGCGCTGCACCAGGTGTGATTCAGGTGAAGT  480
 105  T  H  W  N  D  L  L  F  C  L  R  C  T  R  C  D  S  G  E  V  124
 481 GGAGCTAAGTCCCTGCACCACGACCAGAAACACAGTGTGTCAGTGCGAAGAAGGCACCTT  540
 125  E  L  S  P  C  T  T  T  R  N  T  V  C  Q  C  E  E  G  T  F  144
 541 CCGGGAAGAAGATTCTCCTGAGATGTGCCGGAAGTGCCGCACAGGGTGTCCCAGAGGGAT  600
 145  R  E  E  D  S  P  E  M  C  R  K  C  R  T  G  C  P  R  G  M  164
 601 GGTCAAGGTCGGTGATTGTACACCCTGGAGTGACATCGAATGTGTCCACAAAGAATCAGG  660
 165  V  K  V  G  D  C  T  P  W  S  D  I  E  C  V  H  K  E  S  G  184
 661 CATCATCATAGGAGTCACAGTTGCAGCCGTAGTCTTGATTGTGGCTGTGTTTGTTTGCAA  720
 185  I  I  I  G  V  T  V  A  A  V  V  L  I  V  A  V  F  V  C  K  204
 721 GTCTTTACTGTGGAAGAAAGTCCTTCCTTACCTGAAAGGCATCTGCTCAGGTGGTGGTGG  780
 205  S  L  L  W  K  K  V  L  P  Y  L  K  G  I  C  S  G  G  G  G  224
 781 GGACCCTGAGCGTGTGGACAGAAGCTCACAACGACCTGGGGCTGAGGACAATGTCCTCAA  840
 225  D  P  E  R  V  D  R  S  S  Q  R  P  G  A  E  D  N  V  L  N  244
 841 TGAGATCGTGAGTATCTTGCAGCCCACCCAGGTCCCTGAGCAGGAAATGGAAGTCCAGGA  900
 245  E  I  V  S  I  L  Q  P  T  Q  V  P  E  Q  E  M  E  V  Q  E  264
 901 GCCAGCAGAGCCAACAGGTGTCAACATGTTGTCCCCCGGGGAGTCAGAGCATCTGCTGGA  960
 265  P  A  E  P  T  G  V  N  M  L  S  P  G  E  S  E  H  L  L  E  284
 961 ACCGGCAGAAGCTGAAAGGTCTCAGAGGAGGAGGCTGCTGGTTCCAGCAAATGAAGGTGA 1020
 285  P  A  E  A  E  R  S  Q  R  R  R  L  L  V  P  A  N  E  G  D  304
1021 TCCCACTGAGACTCTGAGACAGTGCTTCGATGACTTTGCAGACTTGGTGCCCTTTGACTC 1080
 305  P  T  E  T  L  R  Q  C  F  D  D  F  A  D  L  V  P  F  D  S  324
1081 CTGGGAGCCGCTCATGAGGAAGTTGGGCCTCATGGACAATGAGATAAAGGTGGCTAAAGC 1140
 325  W  E  P  L  M  R  K  L  G  L  M  D  N  E  I  K  V  A  K  A  344
1141 TGAGGCAGCGGGCCACAGGGACACCTTGTACACGATGCTGATAAAGTGGGTCAACAAAAC 1200
 345  E  A  A  G  H  R  D  T  L  Y  T  M  L  I  K  W  V  N  K  T  364
1201 CGGGCGAGATGCCTCTGTCCACACCCTGCTGGATGCCTTGGAGACGCTGGGAGAGAGACT 1260
 365  G  R  D  A  S  V  H  T  L  L  D  A  L  E  T  L  G  E  R  L  384
1261 TGCCAAGCAGAAGATTGAGGACCACTTGTTGAGCTCTGGAAAGTTCATGTATCTAGAAGG 1320
 385  A  K  Q  K  I  E  D  H  L  L  S  S  G  K  F  M  Y  L  E  G  404
1321 TAATGCAGACTCTGCCATGTCCTAAGTGTGATTCTCTTCAGGAAGTCAGACCTTCCCTGG 1380
 405  N  A  D  S  A  M  S  *                                       424
1381 TTTACCTTTTTTCTGGAAAAAGCCCAACTGGACTCCAGTCAGTAGGAAAGTGCCACAATT 1440
1441 GTCACATGACCGGTACTGGAAGAAACTCTCCCATCCAACATCACCCAGTGGATGGAACAT 1500
1501 CCTGTAACTTTTCACTGCACTTGGCATTATTTTTATAAGCTGAATGTGATAATAAGGACA 1560
1561 CTATGGAAATGTCTGGATCATTCCGTTTGTGCGTACTTTGAGATTTGGTTTGGGATGTCA 1620
1621 TTGTTTTCACAGCACTTTTTTATCCTAATGTAAATGCTTTATTTATTTATTTGGGGTACA 1680
1681 TTGTAAGATCCATCTACACAGTCGTAAAAAAAAAAA                         1717
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FIG. 2

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   1  ATGGCGCCACCACCAGCTAGAGTACATCTAGGTGCGTTCCTGGCAGTGACTCCGAATCCC   60
  61  GGGAGCGCAGCGAGTGGGACAGAGGCAGCCGCGGCCACACCCAGCAAAGTGTGGGGCTCT  120
 121  TCCGCGGGGAGGATTGAACCACGAGGCGGGGGCCGAGGAGCGCTCCCTACCTCCATGGGA  180
 -17                                                        M  G    2
 181  CAGCACGGACCCAGTGCCCGGGCCCGGGCAGGGCGCGCCCCAGGACCCAGGCCGGCGCGG  240
   3  Q  H  G  P  S  A  R  A  R  A  G  R  A  P  G  P  R  P  A  R    22
 241  GAAGCCAGCCCTCGGCTCCGGGTCCACAAGACCTTCAAGTTTGTCGTCGTCGGGGTCCTG  300
  23  E  A  S  P  R  L  R  V  H  K  T  F  K  F  V  V  V  G  V  L    42
 301  CTGCAGGTCGTACCTAGCTCAGCTGCAACCATCAAACTTCATGATCAATCAATTGGCACA  360
  43  L  Q  V  V  P  S  S  A  A  T  I  K  L  H  D  Q  S  I  G  T    62
 361  CAGCAATGGGAACATAGCCCTTTGGGAGAGTTGTGTCCACCAGGATCTCATAGATCAGAA  420
  63  Q  Q  W  E  H  S  P  L  G  E  L  C  P  P  G  S  H  R  S  E    82
 421  CGTCCTGGAGCCTGTAACCGGTGCACAGAGGGTGTGGGTTACACCAATGCTTCCAACAAT  480
  83  R  P  G  A  C  N  R  C  T  E  G  V  G  Y  T  N  A  S  N  N   102
 481  TTGTTTGCTTGCCTCCCATGTACAGCTTGTAAATCAGATGAAGAAGAGAGAAGTCCCTGC  540
 103  L  F  A  C  L  P  C  T  A  C  K  S  D  E  E  E  R  S  P  C   122
 541  ACCACGACCAGGAACACAGCATGTCAGTGCAAACCAGGAACTTTCCGGAATGACAATTCT  600
 123  T  T  T  R  N  T  A  C  Q  C  K  P  G  T  F  R  N  D  N  S   142
 601  GCTGAGATGTGCCGGAAGTGCAGCACAGGGTGCCCCAGAGGGATGGTCAAGGTCAAGGAT  660
 143  A  E  M  C  R  K  C  S  T  G  C  P  R  G  M  V  K  V  K  D   162
 661  TGTACGCCCTGGAGTGACATCGAGTGTGTCCACAAAGAATCAGGCAATGGACATAATATA  720
 163  C  T  P  W  S  D  I  E  C  V  H  K  E  S  G  N  G  H  N  I   182
 721  TGGGTGATTTTGGTTGTGACTTTGGTTGTTCCGTTGCTGTTGGTGGCTGTGCTGATTGTC  780
 183  W  V  I  L  V  V  T  L  V  V  P  L  L  L  V  A  V  L  I  V   202
 781  TGTTGTTGCATCGGCTCAGGTTGTGGAGGGGACCCCAAGTGCATGGACAGGGTGTGTTTC  840
 203  C  C  C  I  G  S  G  C  G  G  D  P  K  C  M  D  R  V  C  F   222
 841  TGGCGCTTGGGTCTCCTACGAGGGCCTGGGGCTGAGGACAATGCTCACAACGAGATTCTG  900
 223  W  R  L  G  L  L  R  G  P  G  A  E  D  N  A  H  N  E  I  L   242
 901  AGCAACGCAGACTCGCTGTCCACTTTCGTCTCTGAGCAGCAAATGGAAAGCCAGGAGCCG  960
 243  S  N  A  D  S  L  S  T  F  V  S  E  Q  Q  M  E  S  Q  E  P   262
 961  GCAGATTTGACAGGTGTCACTGTACAGTCCCCAGGGGAGGCACAGTGTCTGCTGGGACCG 1020
 263  A  D  L  T  G  V  T  V  Q  S  P  G  E  A  Q  C  L  L  G  P   282
1021  GCAGAAGCTGAAGGGTCTCAGAGGAGGAGGCTGCTGGTTCCAGCAAATGGTGCTGACCCC 1080
 283  A  E  A  E  G  S  Q  R  R  R  L  L  V  P  A  N  G  A  D  P   302
1081  ACTGAGACTCTGATGCTGTTCTTTGACAAGTTTGCAAACATCGTGCCCTTTGACTCCTGG 1140
 303  T  E  T  L  M  L  F  F  D  K  F  A  N  I  V  P  F  D  S  W   322
1141  GACCAGCTCATGAGGCAGCTGGACCTCACGAAAAATGAGATCGATGTGGTCAGAGCTGGT 1200
 323  D  Q  L  M  R  Q  L  D  L  T  K  N  E  I  D  V  V  R  A  G   342
1201  ACAGCAGGCCCAGGGGATGCCTTGTATGCAATGCTGATGAAATGGGTCAACAAAACTGGA 1260
 343  T  A  G  P  G  D  A  L  Y  A  M  L  M  K  W  V  N  K  T  G   362
1261  CGGAACGCCTCGATCCACACCCTGCTGGATGCCTTGGAGAGGATGGAAGAGAGACATGCA 1320
 363  R  N  A  S  I  H  T  L  L  D  A  L  E  R  M  E  E  R  H  A   382
1321  AAAGAGAAGATTCAGGACCTCTTGGTGGACTCTGGAAAGTTCATCTACTTAGAAGATGGC 1380
 383  K  E  K  I  Q  D  L  L  V  D  S  G  K  F  I  Y  L  E  D  G   402
1381  ACAGGCTCTGCCGTGTCCTTGGAGTGA                                  1407
 403  T  G  S  A  V  S  L  E  *                                     411
```

FIG. 3

5/ 23

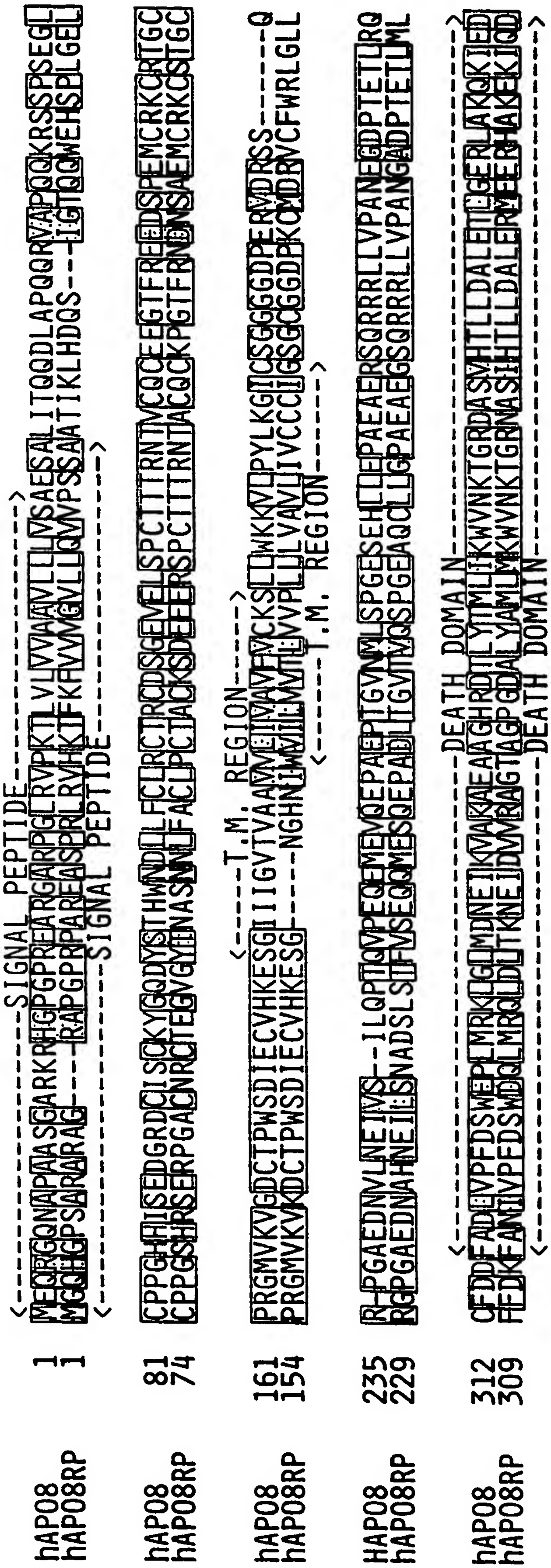

FIG. 4A

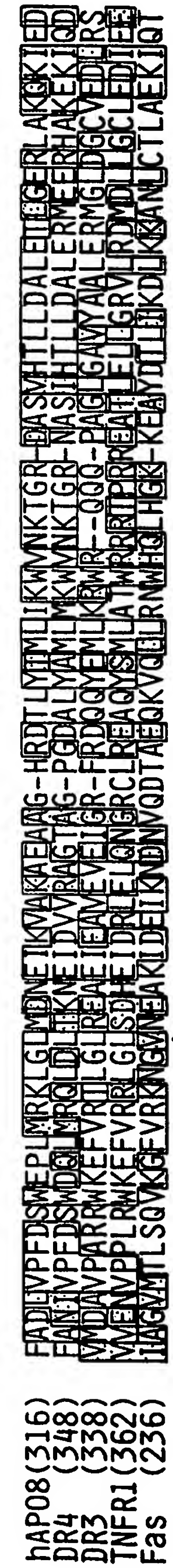

FIG. 4B

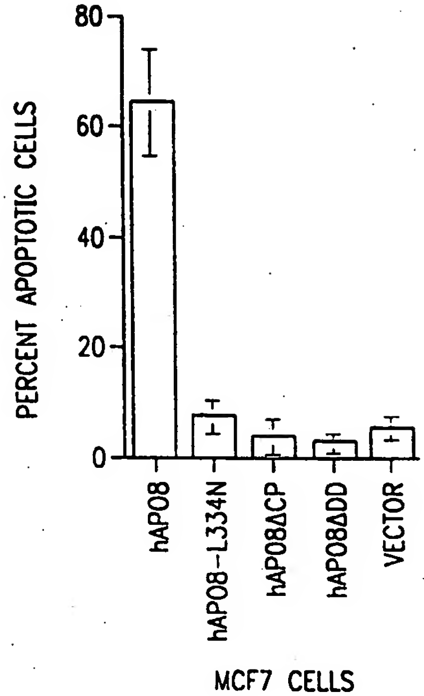

FIG. 5A

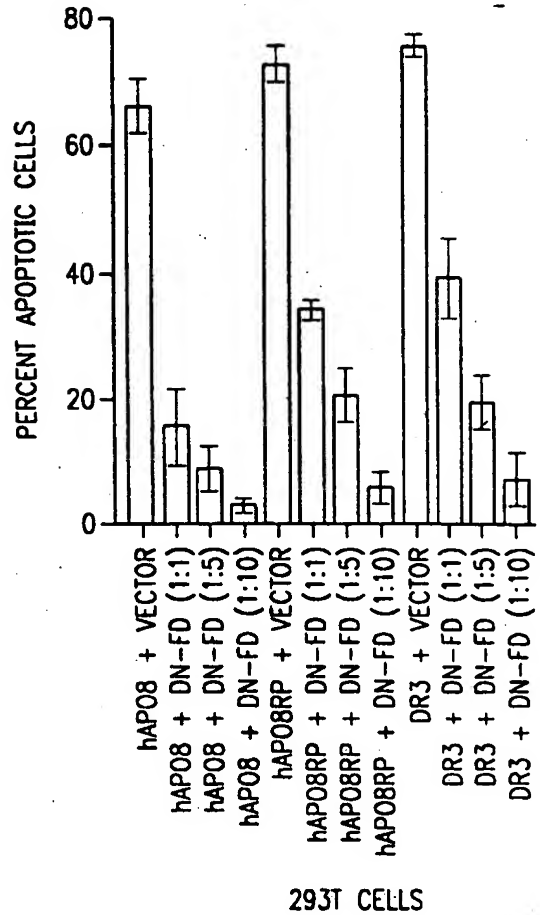

FIG. 5B

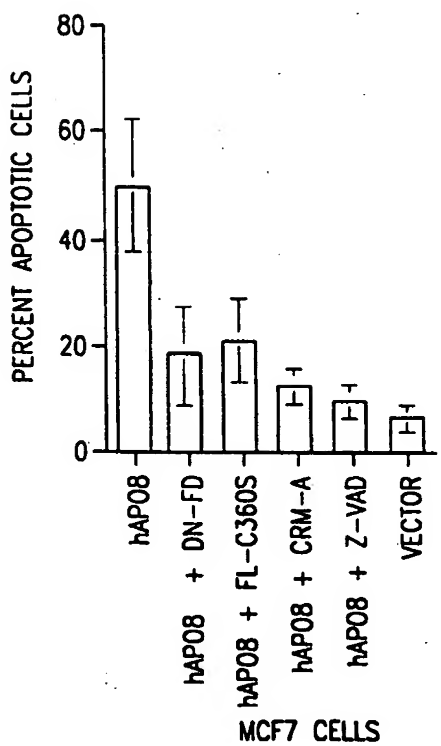

FIG. 5C

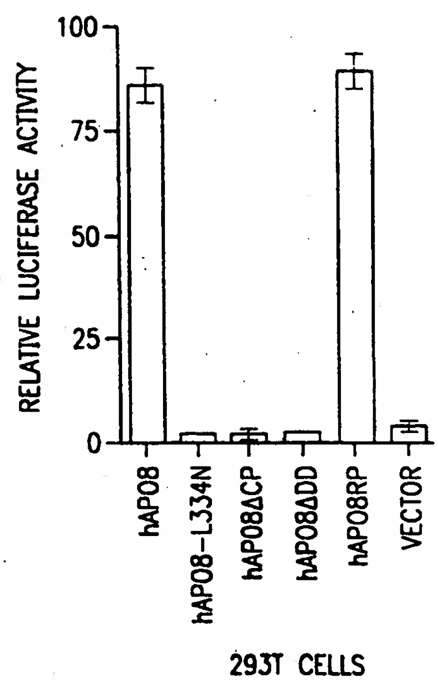

FIG. 5D

```
   1  CACGCGCACGAACTCAGCCAACGATTTCTGATAGATTTTTGGGAGTTTGACCAGAGATGC    60
  61  AAGGGGTGAAGGAGCGCTTCCTACCGTTAGGAACTCTGGGGACAGAGCGCCCCGGCCGCC   120
 121  TGATGGCCGAGGCAGGGTGCGACCCAGGACCCAGGACGGCGTCGGGAACCATACCATGGC   180
 -17                                                          M  A     2
 181  CCGGATCCCCAAGACCCTAAAGTTCGTCGTCGTCATCGTCGCGGTCCTGCTGCCAGTCCT   240
   3   R  I  P  K  T  L  K  F  V  V  V  I  V  A  V  L  L  P  V  L     22
 241  AGCTTACTCTGCCACCACTGCCCGGCAGGAGGAAGTTCCCCAGCAGACAGTGGCCCCACA   300
  23   A  Y  S  A  T  T  A  R  Q  E  E  V  P  Q  Q  T  V  A  P  Q     42
 301  GCAACAGAGGCACAGCTTCAAGGGGGAGGAGTGTCCAGCAGGATCTCATAGATCAGAACA   360
  43   Q  Q  R  H  S  F  K  G  E  E  C  P  A  G  S  H  R  S  E  H     62
 361  TACTGGAGCCTGTAACCCGTGCACAGAGGGTGTGGATTACACCAACGCTTCCAACAATGA   420
  63   T  G  A  C  N  P  C  T  E  G  V  D  Y  T  N  A  S  N  N  E     82
 421  ACCTTCTTGCTTCCCATGTACAGTTTGTAAATCAGATCAAAAACATAAAAGTTCCTGCAC   480
  83   P  S  C  F  P  C  T  V  C  K  S  D  Q  K  H  K  S  S  C  T    102
 481  CATGACCAGAGACACAGTGTGTCAGTGTAAAGAAGGCACCTTCCGGAATGAAAACTCCCC   540
 103   M  T  R  D  T  V  C  Q  C  K  E  G  T  F  R  N  E  N  S  P    122
 541  AGAGATGTGCCGGAAGTGTAGCAGGTGCCCTAGTGGGGAAGTCCAAGTCAGTAATTGTAC   600
 123   E  M  C  R  K  C  S  R  C  P  S  G  E  V  Q  V  S  N  C  T    142
 601  GTCCTGGGATGATATCCAGTGTGTTGAAGAATTTGGTGCCAATGCCACTGTGGAAACCCC   660
 143   S  W  D  D  I  Q  C  V  E  E  F  G  A  N  A  T  V  E  T  P    162
 661  AGCTGCTGAAGAGACAATGAACACCAGCCCGGGGACTCCTGCCCCAGCTGCTGAAGAGAC   720
 163   A  A  E  E  T  M  N  T  S  P  G  T  P  A  P  A  A  E  E  T    182
 721  AATGAACACCAGCCCAGGGACTCCTGCCCCAGCTGCTGAAGAGACAATGACCACCAGCCC   780
 183   M  N  T  S  P  G  T  P  A  P  A  A  E  E  T  M  T  T  S  P    202
 781  GGGGACTCCTGCCCCAGCTGCTGAAGAGACAATGACCACCAGCCCGGGGACTCCTGCCCC   840
 203   G  T  P  A  P  A  A  E  E  T  M  T  T  S  P  G  T  P  A  P    222
 841  AGCTGCTGAAGAGACAATGACCACCAGCCCGGGGACTCCTGCCTCTTCTCATTACCTCTC   900
 223   A  A  E  E  T  M  T  T  S  P  G  T  P  A  S  S  H  Y  L  S    242
 901  ATGCACCATCGTAGGGATCATAGTTCTAATTGTGCTTCTGATTGTGTTTGTTTGAAAGAC   960
 243   C  T  I  V  G  I  I  V  L  I  V  L  L  I  V  F  V  *          262
 961  TTCACTGTGGAAGAAATTCCTTCCTTACCTGAAAGGTTCACGTTACGCGCTGGCTGAAGG  1020
1021  CGGGGGGCGCTGGACACTCTCTGCCCTGCCTCCCTCTGCTGTGTTCCCACAGACAGAAAC  1080
1081  GCCTGCCCCTGCCCCAAGTCCTGGTGTCTCCAGCCTGGCTCTATCTTCCTCCTTGTGATC  1140
1141  GTCCCATCCCCACATCCCGTGCACCCCCCAGGACCCTGGTCTCATCAGTCCCTCTCCTGG  1200
1201  AGCTGGGGGTCCACACATCTCCCAGCCAAGTCCAAGAGGGCAGGGCCAGTTCCTCCCATC  1260
1261  TTCAGGCCCAGCCAGGCAGGGGGCAGTCGGCTCCTCAACTGGGTGACAAGGGTGAGGATG  1320
1321  AGAAGTGGTCACGGGATTTATTCAGCCTTGGTCAGAGCAGAAAAA                 1365
```

FIG. 6

```
   1 CCAGCCTCAAACTGCAGTCCGGCGCCGCGGGGCAGGACAAGGGGAAGGAATAAACACGTT   60
  61 TGGTGAGAGCCATGGCACTCAAGGTCCTACCTCTACACAGGACGGTGCTCTTCGCTGCCA  120
  -2             M  A  L  K  V  L  P  L  H  R  T  V  L  F  A  A  I   17
 121 TTCTCTTCCTACTCCACCTGGCATGTAAAGTGAGTTGCGAAACCGGAGATTGCAGGCAGC  180
  18  L  F  L  L  H  L  A  C  K  V  S  C  E  T  G  D  C  R  Q  Q   37
 181 AGGAATTCAAGGATCGATCTGGAAACTGTGTCCTCTGCAAACAGTGCGGACCTGGCATGG  240
  38  E  F  K  D  R  S  G  N  C  V  L  C  K  Q  C  G  P  G  M  E   57
 241 AGTTGTCCAAGGAATGTGGCTTCGGCTATGGGGAGGATGCACAGTGTGTGCCCTGCAGGC  300
  58  L  S  K  E  C  G  F  G  Y  G  E  D  A  Q  C  V  P  C  R  P   77
 301 CGCACCGGTTCAAGGAAGACTGGGGTTTCCAGAAGTGTAAGCCATGTGCGGACTGTGCGC  360
  78  H  R  F  K  E  D  W  G  F  Q  K  C  K  P  C  A  D  C  A  L   97
 361 TGGTGAACCGCTTTCAGAGGGCCAACTGCTCACACACCAGTGATGCTGTCTGCGGGGACT  420
  98  V  N  R  F  Q  R  A  N  C  S  H  T  S  D  A  V  C  G  D  C  117
 421 GCCTGCCAGGATTTTACCGGAAGACCAAACTGGTTGGTTTTCAAGACATGGAGTGTGTGC  480
 118  L  P  G  F  Y  R  K  T  K  L  V  G  F  Q  D  M  E  C  V  P  137
 481 CCTGCGGAGACCCACCTCCTCCCTACGAACCACACTGTACCAGCAAGGTGAACCTTGTGA  540
 138  C  G  D  P  P  P  P  Y  E  P  H  C  T  S  K  V  N  L  V  K  157
 541 AGATCTCCTCCACCGTCTCCAGCCCTCGGGACACGGCGCTGGCTGCCGTCATCTGCAGTG  600
 158  I  S  S  T  V  S  S  P  R  D  T  A  L  A  A  V  I  C  S  A  177
 601 CTCTGGCCACGGTGCTGCTCGCCCTGCTCATCCTGTGTGTCATCTACTGCAAGAGGCAGT  660
 178  L  A  T  V  L  L  A  L  L  I  L  C  V  I  Y  C  K  R  Q  F  197
 661 TCATGGAGAAGAAACCCAGCTGGTCTCTGCGGTCACAGGACATTCAGTACAATGGCTCTG  720
 198  M  E  K  K  P  S  W  S  L  R  S  Q  D  I  Q  Y  N  G  S  E  217
 721 AGCTGTCATGCTTTGACCAGCCTCGGCTCCGCCACTGTGCCCATAGAGCATGCTGTCAGT  780
 218  L  S  C  F  D  Q  P  R  L  R  H  C  A  H  R  A  C  C  Q  Y  237
 781 ATCACCGGGACTCAGCCCCAATGTATGGGCCTGTTCACCTGATTCCGTCCTTGTGCTGTG  840
 238  H  R  D  S  A  P  M  Y  G  P  V  H  L  I  P  S  L  C  C  E  257
 841 AAGAGGCCCGCAGCTCTGCCCGAGCTGTGCTTGGCTGTGGGCTGCGTTCTCCCACTACCC  900
 258  E  A  R  S  S  A  R  A  V  L  G  C  G  L  R  S  P  T  T  L  277
 901 TCCAGGAGAGAAACCCGGCTTCTGTGGGGAACACGATGCCAGCCTTCTTTGGGTCTGTTT  960
 278  Q  E  R  N  P  A  S  V  G  N  T  M  P  A  F  F  G  S  V  S  297
 961 CCCGTTCCATCTGCGCGGAGTTTTCTGATGCCTGGCCTCTGATGCAGAATCCTCTGGGCG 1020
 298  R  S  I  C  A  E  F  S  D  A  W  P  L  M  Q  N  P  L  G  G  317
1021 GTGACAGCTCTCTCTGTGACTCTTATCCTGAACTCACTGGAGAAGATACCAATTCCCTCA 1080
 318  D  S  S  L  C  D  S  Y  P  E  L  T  G  E  D  T  N  S  L  N  337
1081 ATCCCGAAAACGAAAGCACAGCATCTCTGGATTCCAGTGGCGGCCAGGATCTGGCTGGGA 1140
 338  P  E  N  E  S  T  A  S  L  D  S  S  G  G  Q  D  L  A  G  T  357
1141 CAGCTGCTCTAGAGTCTTCTGGGAATGTTTCAGAATCTACTGACTCACCTAGACATGGTG 1200
 358  A  A  L  E  S  S  G  N  V  S  E  S  T  D  S  P  R  H  G  D  377
1201 ACACTGGTACAGTCTGGGAGCAGACGCTAGCTCAGGATGCTCAAAGGACTCCAAGCCAAG 1260
 378  T  G  T  V  W  E  Q  T  L  A  Q  D  A  Q  R  T  P  S  Q  G  397
1261 GAGGCTGGGAAGACAGGGAAAACCTGAATCTAGCCATGCCCACAGCCTTCCAGGATGCCT 1320
 398  G  W  E  D  R  E  N  L  N  L  A  M  P  T  A  F  Q  D  A  *  417
1321 GAAGGCCATCTTCCTGACGTGGAGGTGTGGGTCTGGACACGCCTGTGATGAGGCCTACAG 1380
1381 ACTGAGCAGTCTTGGTGTCTGGAAGCAAAAATAAATCTGAACCAAACTGACAACATTTCC 1440
1441 ATCCTTTCAGCCACTAGCTACTGAGCCAGACCAGCTGTAAGCTGAAACCCCAGCAAGAAG 1500
1501 CAAGGAGAGACTTGACTGTAGGCGGCCTTGGGACATGTGCTTCTTCCCTAAGCGAAAACC 1560
1561 TTAGCTGGGGCCAATTTGAAGGACCCATGGGTGGAATGTGCTGCCTGTGAACTTGTGGGC 1620
1621 ACAGCAGGACCCAGCCTGGCTCCTTCTTATGTCCACGGTGAATGTGGTTTCACAAGAC   1678
```

FIG. 7A

```
  1  CCAGCCTCAAACTGCAGTCCGGCGCCGCGGGGCAGGACAAGGGGAAGGAATAAACACGTT  60
 61  TGGTGAGAGCCATGGCACTCAAGGTCCTACCTCTACACAGGACGGTGCTCTTCGCTGCCA  120
 -2              M  A  L  K  V  L  P  L  H  R  T  V  L  F  A  I   17
121  TTCTCTTCCTACTCCACCTGGCATGTAAAGTGAGTTGCGAAACCGGAGATTGCAGGCAGC  180
 18    L  F  L  L  H  L  A  C  K  V  S  C  E  T  G  D  C  R  Q  Q   37
181  AGGAATTCAAGGATCGATCTGGAAACTGTGTCCTCTGCAAACAGTGCGGACCTGGCATGG  240
 37    E  F  K  D  R  S  G  N  C  V  L  C  K  Q  C  G  P  G  M  E   57
241  AGTTGTCCAAGGAATGTGGCTTCGGCTATGGGGAGGATGCACAGTGTGTGCCCTGCAGGC  300
 58    L  S  K  E  C  G  F  G  Y  G  E  D  A  Q  C  V  P  C  R  P   77
301  CGCACCGGTTCAAGGAAGACTGGGGTTTCCAGAAGTGTAAGCCATGTGCGGACTGTGCGC  360
 78    H  R  F  K  E  D  Q  G  F  Q  K  C  K  P  C  A  D  C  A  L   97
361  TGGTGAACCGCTTTCAGAGGGCCAACTGCTCACACACCAGTGATGCTGTCTGCGGGGACT  420
 98    V  N  R  F  Q  R  A  N  C  S  H  T  S  D  A  V  C  G  D  C  117
421  GCCTGCCAGGATTTTACCGGAAGACCAAACTGGTTGGTTTTCAAGACATGGAGTGTGTGC  480
118    L  P  G  F  Y  R  K  T  K  L  V  G  F  Q  D  M  E  C  V  P  137
481  CCTGCGGAGACCCACCTCCTCCCTACGAACCACACTGTACCAGCAAGGTGAACCTTGTGA  540
138    C  G  D  P  P  P  P  Y  E  P  H  C  T  S  K  V  N  L  V  K  157
541  AGATCTCCTCCACCGTCTCCAGCCCTCGGGACACGGCGCTGGCTGCCGTCATCTGCAGTG  600
158    I  S  S  T  V  S  S  P  R  D  T  A  L  A  A  V  I  C  S  A  177
601  CTCTGGCCACGGTGCTGCTCGCCCTGCTCATCCTGTGTGTCATCTACTGCAAGAGGCAGT  660
178    L  A  T  V  L  L  A  L  L  I  L  C  V  I  Y  C  K  R  Q  F  197
661  TCATGGAGAAGAAACCCAGCTGTAAGCTCCCATCCCTCTGTCTCACTGTGAAGTGAGCTT  720
198    M  E  K  K  P  S  C  K  L  P  S  L  C  L  T  V  K  *         217
721  GTTAGCATTGTCACCCAAGAGTTCTCAAGACACCTGGCTGAGACCTAAGACCTTTAGAGC  780
781  ATCAACAGCTACTTAGAATACAAGATGCAGGAAAACGAGCCTCTTCAGGAATCTCAGGGC  840
841  CTCCTAGGGATGCTGGCAAGGCTGTGATGTCTCAAGGCTACCAGGA                886
```

FIG. 7B

```
1     GGACCTGCAGCCTCCCAGGTGGCTGGGAAGAACTCTCCAACAATAAATACATTTGATAAG  60
61    AAAGATGGCTTTAAAAGTGCTACTAGAACAAgAGAAAACGTTTTTCACTCTTTTAGTATT  120
0        M  A  L  K  V  L  L  E  Q  E  K  T  F  F  T  L  L  V  L    19
121   ACTAGGCTATTTGTCATGTAAAGTGACTTGTGAATCAGGAGACTGTAGACAGCAAGAATT  180
20     L  G  Y  L  S  C  K  V  T  C  E  S  G  D  C  R  Q  Q  E  F   39
181   CAGGGATCGGTCTGGAAACTGTGTTCCCTGCAACCAGTGTGGGCCAGGCATGGAGTTGTC  240
40     R  D  R  S  G  N  C  V  P  C  N  Q  C  G  P  G  M  E  L  S   59
241   TAAGGAATGTGGCTTCGGCTATGGGGAGGATGCACAGTGTGTGGCGTGCCGGCTGCACAG  300
60     K  E  C  G  F  G  Y  G  E  D  A  Q  C  V  A  C  R  L  H  R   79
301   GTTCAAGGAGGACTGGGGCTTCCAGAAATGCAAGCCCTGTCTGGACTGCGCAGTGGTGAA  360
80     F  K  E  D  W  G  F  Q  K  C  K  P  C  L  D  C  A  V  V  N   99
361   CCGCTTTCAGAAGGCAAATTGTTCAGCCACCAGTGATGCCATCTGCGGGGACTGCTTGCC  420
100    R  F  Q  K  A  N  C  S  A  T  S  D  A  I  C  G  D  C  L  P   119
421   AGGATTTTATAGGAAGACGAAACTTGTCGGCTTTCAAGACATGGAGTGTGTGCCTTGTGG  480
120    G  F  Y  R  K  T  K  L  V  G  F  Q  D  M  E  C  V  P  C  G   139
481   AGACCCTCCTCCTCCTTACGAACCGCACTGTGCCAGCAAGGTCAACCTCGTGAAGATCGC  540
140    D  P  P  P  P  Y  E  P  H  C  A  S  K  V  N  L  V  K  I  A   159
541   GTCCACGGCCTCCAGCCCACGGGACACGGCGCTGGCTGCCGTTATCTGCAGCGCTCTGGC  600
160    S  T  A  S  S  P  R  D  T  A  L  A  A  V  I  C  S  A  L  A   179
601   CACCGTCCTGCTGGCCCTGCTCATCCTCTGTGTCATCTATTGTAAGAGACAGTTTATGGA  660
180    T  V  L  L  A  L  L  I  L  C  V  I  Y  C  K  R  Q  F  M  E   199
661   AAAGAAACCCAGCTGGTCTCTGCGGTCACAGGACATTCAGTACAACGAgactGAGCTGTC  720
200    K  K  P  S  W  S  L  R  S  Q  D  I  Q  Y  N  E  T  E  L  S   219
721   GTGTTTTGACAGACCTCAGCTCCACGAATATGCCCACAGAGCCTGCTGCCAGTGCCGCCG  780
220    C  F  D  R  P  Q  L  H  E  Y  A  H  R  A  C  C  Q  C  R  R   239
781   TGACTCAGTGCAGACCTGCGGGCCGGTGCGCTTGCTCCCATCCATGTGCTGTGAGGAGGC  840
240    D  S  V  Q  T  C  G  P  V  R  L  L  P  S  M  C  C  E  E  A   259
841   CTGCAGCCCCAACCCGGCGACTCTTGGTTGTGGGGTGCATTCTGCAGCCAGTCTTCAGGC  900
260    C  S  P  N  P  A  T  L  G  C  G  V  H  S  A  A  S  L  Q  A   279
901   AAGAAACGCAGGCCCAGCCGGGGAGATGGTGCCGACTTTCTTCGGATCCCTCACGCAGTC  960
280    R  N  A  G  P  A  G  E  M  V  P  T  F  F  G  S  L  T  Q  S   299
961   CATCTGTGGCGAGTTTTCAGATGCCTGGCCTCTGATGCAGAATCCCATGGGTGGTGACAA  1020
300    I  C  G  E  F  S  D  A  W  P  L  M  Q  N  P  M  G  G  D  N   319
1021  CATCTCTTTTTGTGACTCTTATCCTGAACTCACTGGAGAAGACATTCATTCTCTCAATCC  1080
320    I  S  F  C  D  S  Y  P  E  L  T  G  E  D  I  H  S  L  N  P   339
1081  AGAACTTGAAAGCTCAACGTCTTTGGATTCAAATAGCAGTCAAGATTTGGTTGGTGGGGC  1140
340    E  L  E  S  S  T  S  L  D  S  N  S  S  Q  D  L  V  G  G  A   359
1141  TGTTCCAGTCCAGTCTCATTCTGAAAACTTTACAGCAGCTACTGATTTATCTAGATATAA  1200
360    V  P  V  Q  S  H  S  E  N  F  T  A  A  T  D  L  S  R  Y  N   379
1201  CAACACACTGGTAGAATCAGCATCAACTCAGGATGCACTAACTATGAGAAGCCAGCTAGA  1260
380    N  T  L  V  E  S  A  S  T  Q  D  A  L  T  M  R  S  Q  L  D   399
1261  TCAGGAGAGTGGCGCTGTCATCCACCCAGCCACTCAGACGTCCCTCCAGGTAAGGCAGCG  1320
400    Q  E  S  G  A  V  I  H  P  A  T  Q  T  S  L  Q  V  R  Q  R   419
1321  ACTGGGTTCCCTGTGAACACAGCACTGACTTACAGTAGATCAGAACTCTGTTCCCAGCAT  1380
420    L  G  S  L  *                                                439
1381  AAGATTTGGGGGAACCTGGATGAGTTTTTTTTTTTGCATCTTTAATAATTTCTTATATGT  1440
1441  TGTAGAGTATGTTTTAAAATAAATTTCAAGTATTTTTTTAAAAAACTTT             1489
```

FIG. 7C

```
1     TGGCCTCTGATGCAGAATCCTCTGGGTGGTGACAGCTCTCTCTGTGACTCTTATCCTGAA  60
1     W  P  L  M  Q  N  P  L  G  G  D  S  S  L  C  D  S  Y  P  E    20
61    CTCACTGGAGAAGATATCAATTCCCTCAATCCCGAAAATGAAAGCTCAACATCTGTGGAT  120
21    L  T  G  E  D  I  N  S  L  N  P  E  N  E  S  S  T  S  V  D    40
121   TCCAATGGTGGTCAGGATCTGGCTGGGGCAGCTGCTCCAGATTCTTCTGGAGATTTTCAA  180
41    S  N  G  G  Q  D  L  A  G  A  A  A  P  D  S  S  G  D  F  Q    60
181   GAAAATACTGACTCACCTAGATATGGTGACGCCGATACGGTCTGGGAGCAAACCCTAGCT  240
61    E  N  T  D  S  P  R  Y  G  D  A  D  T  V  W  E  Q  T  L  A    80
241   CAGGATGCTCAAAGGACTCCAAGCAGAGAGGGCTGGGAAGCCAGTGAAAACCTGAATCTA  300
81    Q  D  A  Q  R  T  P  S  R  E  G  W  E  A  S  E  N  L  N  L    100
301   GCCACGTCCACAGCCTTCCAGGATGCCTGAAGGCCATCTTCCTGACATGGAGGTGTGGGT  360
101   A  T  S  T  A  F  Q  D  A  *
361   CTGGGCACGCCTCTGGTGAGTCTTACAGACTGAGCAGTCTTGACCTTCTACGGTGTCTGG  420
421   AAGGAAAAATAAATCTGAAGCAAACTGGCAGCACTTCCATACTTTCAGCCACGAGCTTCT  480
481   GAGCCAGACCAGCTGTTAGCTGAAACCCCAGCAAGAAGCAAGGAGAGACCGTAGGCGGCC  540
541   TCGGGACCTGCACTGCTTCCCTAAACAAGAACCTTAGCTGGGGCCAATTTGAAGGACCAT  600
601   GATGGAATGTGCTGCCTGTGAGATTGTGGGCACGGCAGGGCCCAGCCTGGCTCCTTCTTA  660
661   CGTTCATGGTGAATGTGGTTTCACAAGAACCAGAGTATAAACTTTCATAGACTTCTCTTT  720
721   TAAACATTACCCACTACCCTGGGAAAAAGCCTTAGCAGATGACTTTCTTAGATTTAGGCA  780
781   GACTCTGGGGGTTCCTGTTGAAAAAAAGCCTTTCTAATCANCAAAATNTTTCTCCCATTG  840
841   GTTTNAAAGGGNCTNCTTACCCCCCCCTGAATTGTTATTTNGGTGAAATTTAANTGGCCT  900
901   GCCCCTTATNCCCCTTCCTTTGGNCNCTCCCCTGTGGGTTTTCNCCNCCCTTNACNGGAA  960
961   AAGAAGCCCGGGTCTTNAAAAGGTGGTTTTTAATNTTGTTTNCAAATTTTCCCGGTTAAG  1020
1021  GGGAAGGAAGATTAAAAGGGGAAAAGGGCTCCCTTTTAANTTTNCCCTNNTCCAATTTCC  1080
1081  TNAAACCTGNNTTTTAACCNTCCCCCGTTAATTTGGAANCTTTTTTATTCCGC         1133
```

FIG. 7D

Nucleotide and amino acid sequence of mAPO4-γ

```
  1  GCGTTTGGCGCGGAAGTGCTACCAAGCTGCGGAAAGCGTGAGTCTGGAGCACAGCACTGG   60
 61  CGAGTAGCAGGAATAAACACGTTTGGTGAGAGCCATGGCACTCAAGGTCCTACCTCTACA  120
-10                                          M  A  L  K  V  L  P  L  H    9
121  CAGGACGGTGCTCTTCGCTGCCATTCTCTTCCTACTCCACCTGGCATGTAAAGTGAGTTG  180
 10   R  T  V  L  F  A  A  I  L  F  L  L  H  L  A  C  K  V  S  C   29
181  CGAAACCGGAGATTGCAGGCAGCAGGAATTCAAGGATCGATCTGGAAACTGTGTCCTCTG  240
 30   E  T  G  D  C  R  Q  Q  E  F  K  D  R  S  G  N  C  V  L  C   49
241  CAAACAGTGCGGACCTGGCATGGAGTTGTCCAAGGAATGTGGCTTCGGCTATGGGGAGGA  300
 50   K  Q  C  G  P  G  M  E  L  S  K  E  C  G  F  G  Y  G  E  D   69
301  TGCACAGTGTGTGCCCTGCAGGCCGCACCGGTTCAAGGAAGACTGGGGTTTCCAGAAGTG  360
 70   A  Q  C  V  P  C  R  P  H  R  F  K  E  D  W  G  F  Q  K  C   89
361  TAAGCCATGTGCGGACTGTGCGCTGGTGAACCGCTTTCAGAGGGCCAACTGCTCACACAC  420
 90   K  P  C  A  D  C  A  L  V  N  R  F  Q  R  A  N  C  S  H  T  109
421  CAGTGATGCTGTCTGCGGGGACTGCCTGCCAGGATTTTACCGGAAGACCAAACTGGTTGG  480
110   S  D  A  V  C  G  D  C  L  P  G  F  Y  R  K  T  K  L  V  G  129
481  TTTTCAAGACATGGAGTGTGTGCCCTGCGGAGACCCACCTCCTCCCTACGAACCACACTG  540
130   F  Q  D  M  E  C  V  P  C  G  D  P  P  P  P  Y  E  P  H  C  149
541  TGAGTGATGTGCCAAGTGGCAGCAGACCTTTAAAAAAAAAAGAAAAAAAAA           591
150   E  *                                                          166
```

FIG. 7E

Nucleotide and amino acid sequence of mAPO4-β

```
  1  CCACGCGTCCGATCCTGAACTCACTGGAGAAGATACCAATTCCCTCAATCCCGAAAACGA   60
  1   H  A  S  D  P  E  L  T  G  E  D  T  N  S  L  N  P  E  N  E    20
 61  AAGCGCAGCATCTCTGGATTCCAGTGGCGGCCAGGATCTGGCTGGGACAGCTGCTCTAGA  120
 21   S  A  A  S  L  D  S  S  G  G  Q  D  L  A  G  T  A  A  L  E    40
121  GTCTTCTGGGAATGTTTCAGAATCTACTGACTCACCTAGACATGGTGACACTGGTACAGT  180
 41   S  S  G  N  V  S  E  S  T  D  S  P  R  H  G  D  T  G  T  V    60
181  CTGGGAGCAGACGCTAGCTCAGGATGCTCAAAGGACTCCAAGCCAAGGAGGCTGGGAAGA  240
 61   W  E  Q  T  L  A  Q  D  A  Q  R  T  P  S  Q  G  G  W  E  D    80
241  CAGGGAAAACCTGAATCTAGCCATGCCCACAGCCTTCCAGGATGCCTGAAGGCCATCTTC  300
 81   R  E  N  L  N  L  A  M  P  T  A  F  Q  D  A  *                95
301  CTGACGTGGAGGTGTGGGTCTGGACAAGCCTGTGATGAGGCCTACAGACTGAGCAGTCTT  360
361  GGTGTCTGGAAGCAAAAATAAATCTGAACCAAACTG                          396
```

FIG. 8

PARTIAL NUCLEOTIDE AND AMINO ACIDS SEQUENCE OF hAPO6

```
  1 TGGAGCGCTGCCGCTACTGCAACGTCCTCTGCGGGGAGCGTGAGGAGGAGGCACGGGCTT  60
  1   E  R  C  R  Y  C  N  V  L  C  G  E  R  E  E  E  A  R  A  C  20
 61 GCCACGCCACCCACAACCGTGCCTGCCGCTGCCGCACCGGCTTCTTCGCGCACGCTGGTT 120
 21   H  A  T  H  N  R  A  C  R  C  R  T  G  F  F  A  H  A  G  F  40
121 TCTGCTTGGAGCACGCATCGTGTCCACCTGGTGCCGGCGTGATTGCCCCGGGCACCCCCA 180
 41   C  L  E  H  A  S  C  P  P  G  A  G  V  I  A  P  G  T  P  S  60
181 GCCAGAACACGCAGTGCCAGCCGTGCCCCCCAGGCACCTTCTCAGCCAGCAGCTCCAGCT 240
 61   Q  N  T  Q  C  Q  P  C  P  P  G  T  F  S  A  S  S  S  S  S  80
241 CAGAGCAGTGCCAGCCCCACCGCAACTGCACGGCCCTGGGCCTGGCCCTCAATGTGCCAG 300
 81   E  Q  C  Q  P  H  R  N  C  T  A  L  G  L  A  L  N  V  P  G 100
301 GCTCTTCCTCCCATGACACCCTGTGCACCAGCTGCACTGGCTTCCCCCTCAGCACCAGGG 360
101   S  S  S  H  D  T  L  C  T  S  C  T  G  F  P  L  S  T  R  V 120
361 TACCAGGAGCTGAGGAGTGTGAGCGTGCCGTCATCGACTTTGTGGCTTTCCAGGACATCT 420
121   P  G  A  E  E  C  E  R  A  V  I  D  F  V  A  F  Q  D  I  S 140
421 CCATCAAGAGGCTGCAGCGGCTGCTGCAGgCCCTCGAGGCCCcGGAGGGCTGGGGTCCGA 480
141   I  K  R  L  Q  R  L  L  Q  A  L  E  A  P  E  G  W  G  P  T 160
481 CACCAAGGGCGGgCCGCGCGGCCTTGcAGCTGAAGCTGCGTCGGCGGCTCACGGAGCTCC 540
161   P  R  A  G  R  A  A  L  Q  L  K  L  R  R  R  L  T  E  L  L 180
541 TGGGGGCGCAGGACGGGGCGCTGCTGGTGCGGCTGCTGCAGGCgctGCGCGTGGCCAGGA 600
181   G  A  Q  D  G  A  L  L  V  R  L  L  Q  A  L  R  V  A  R  M 200
601 TGCCCGGGCTGGAGCGGAGCGTCCGTGAGCGCTTCCTCCCTGTGCACTGATCCTGGCCCC 660
201   P  G  L  E  R  S  V  R  E  R  F  L  P  V  H  *             220
661 CTCTTATTTATTCTACATCCTTGGCACCCCACTTGCACTGAAAGAGGCTTTTTTTTAAAT 720
721 AGAAGAAATGAGGTTTCTTAAAAAAAAAAAAAAAAAAAAAAAAAAAA  767
```

FIG. 9

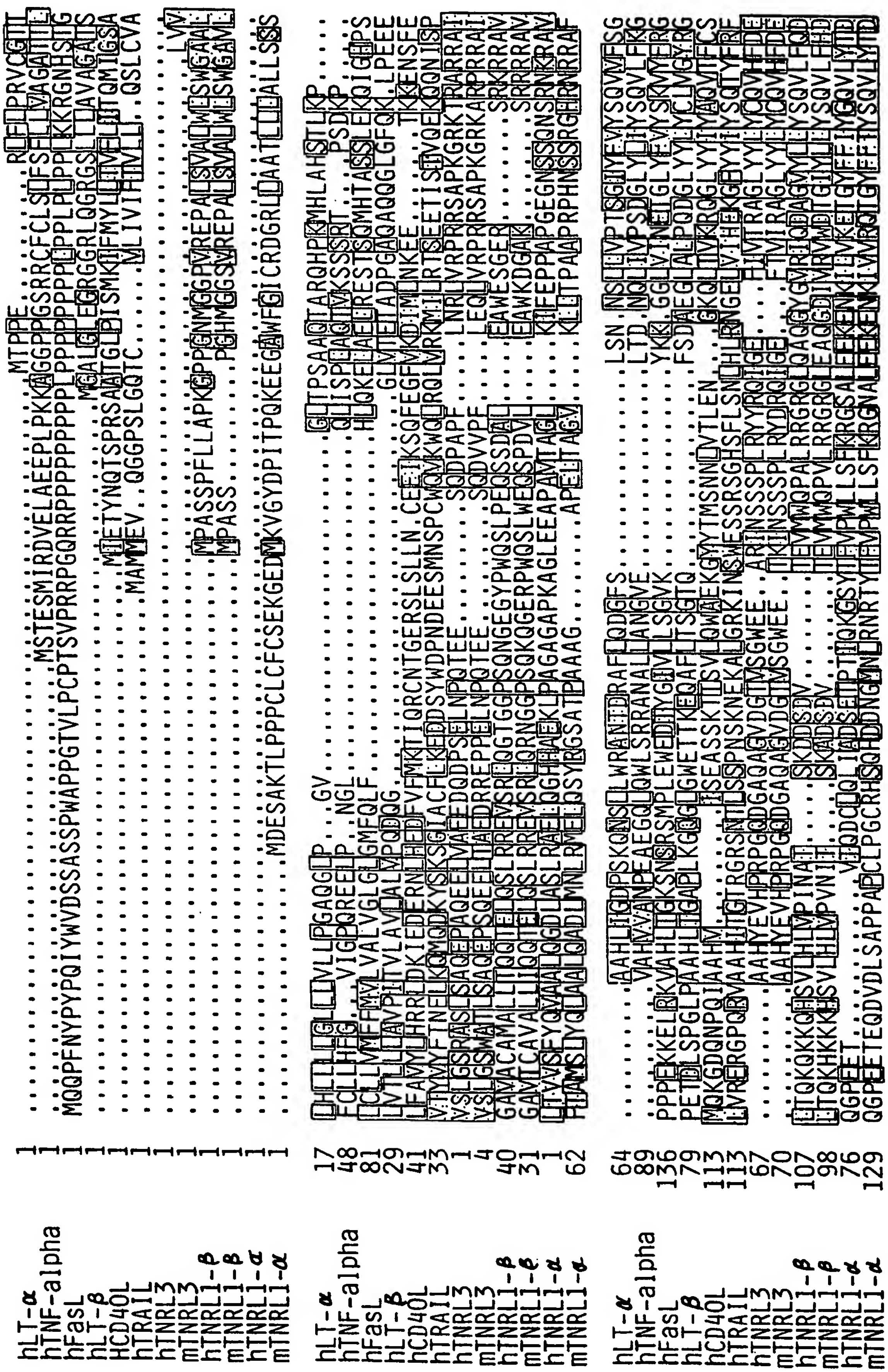

FIG. 10A

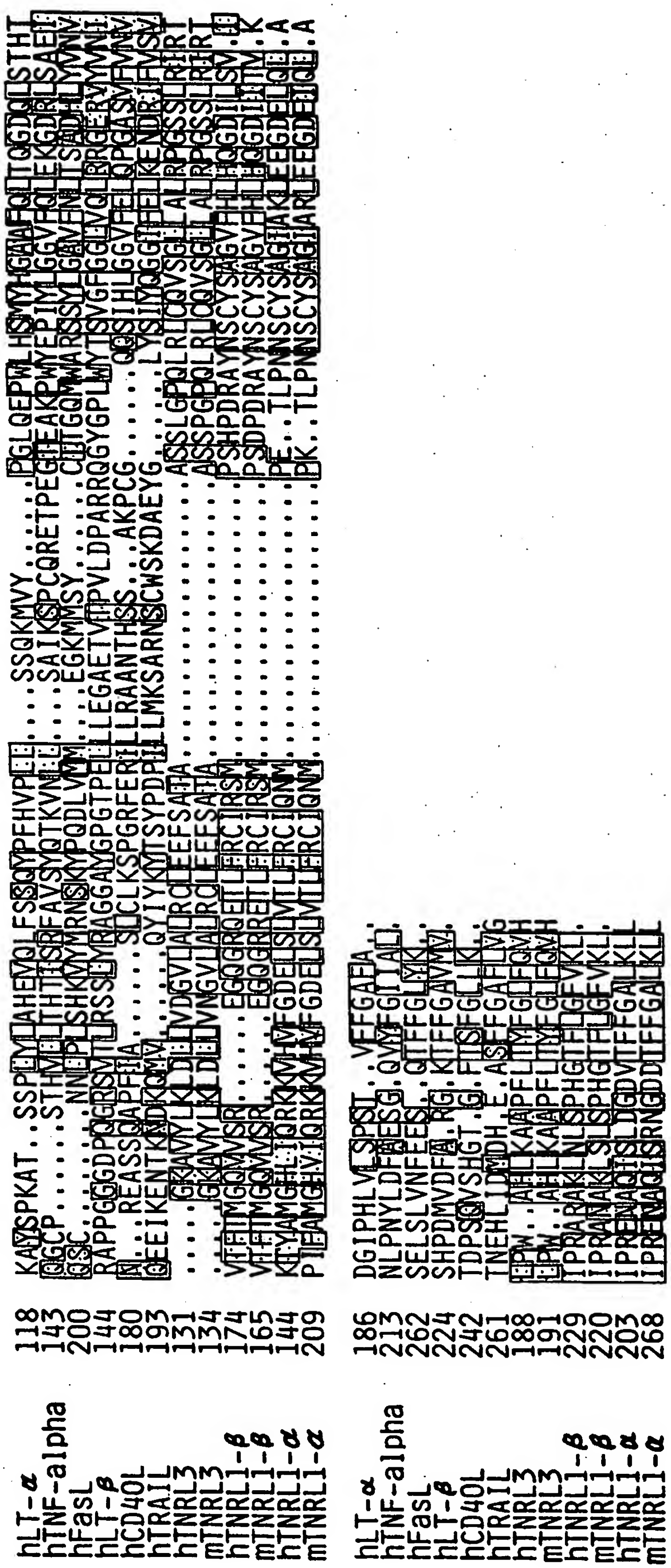

FIG. 10B

NUCLEOTIDE AND AMINO ACID SEQUENCE OF FULL-LENGTH HUMAN TNRL-1 (TNRL-alpha)

```
   1 GAAAGGCAGAAAGGAGAAAATTCAGGATAACTCTCCTGAGGGGTGAGCCAAGCCCTGCCA   60
  61 TGTAGTGCACGCAGGACATCAACAAACACAGATAACAGGAAATGATCCATTCCCTGTGGT  120
 121 CACTATTCTAAAGGCCCCAACCTTCAAAGTTCAAGTAGTGATATGGATGACTCCACAGAA  180
 -13                                                M  D  D  S  T  E     6
 181 AGGGAGCAGTCACGCCTTACTTCTTGCCTTAAGAAAAGAGAAGAAATGAAACTGAAGGAG  240
   7 R  E  Q  S  R  L  T  S  C  L  K  K  R  E  E  M  K  L  K  E     26
 241 TGTGTTTCCATCCTCCCACGGAAGGAAAGCCCCTCTGTCCGATCCTCCAAAGACGGAAAG  300
  27 C  V  S  I  L  P  R  K  E  S  P  S  V  R  S  S  K  D  G  K     46
 301 CTGCTGGCTGCAACCTTGCTGCTGGCACTGCTGTCTTGCTGCCTCACGGTGGTGTCTTTC  360
  47 L  L  A  A  T  L  L  L  A  L  L  S  C  C  L  T  V  V  S  F     66
 361 TACCAGGTGGCCGCCCTGCAAGGGGACCTGGCCAGCCTCCGGGCAGAGCTGCAGGGCCAC  420
  67 Y  Q  V  A  A  L  Q  G  D  L  A  S  L  R  A  E  L  Q  G  H     86
 421 CACGCGGAGAAGCTGCCAGCAGGAGCAGGAGCCCCCAAGGCCGGCCTGGAGGAAGCTCCA  480
  87 H  A  E  K  L  P  A  G  A  G  A  P  K  A  G  L  E  E  A  P    106
 481 GCTGTCACCGCGGGACTGAAAATCTTTGAACCACCAGCTCCAGGAGAAGGCAACTCCAGT  540
 107 A  V  T  A  G  L  K  I  F  E  P  P  A  P  G  E  G  N  S  S    126
 541 CAGAACAGCAGAAATAAGCGTGCCGTTCAGGGTCCAGAAGAAACAGTCACTCAAGACTGC  600
 127 Q  N  S  R  N  K  R  A  V  Q  G  P  E  E  T  V  T  Q  D  C    146
 601 TTGCAACTGATTGCAGACAGTGAAACACCAACTATACAAAAAGGATCTTACACATTTGTT  660
 147 L  Q  L  I  A  D  S  E  T  P  T  I  Q  K  G  S  Y  T  F  V    166
 661 CCATGGCTTCTCAGCTTTAAAAGGGGAAGTGCCCTAGAAGAAAAAGAGAATAAAATATTG  720
 167 P  W  L  L  S  F  K  R  G  S  A  L  E  E  K  E  N  K  I  L    186
 721 GTCAAAGAAACTGGTTACTTTTTTATATATGGTCAGGTTTTATATACTGATAAGACCTAC  780
 187 V  K  E  T  G  Y  F  F  I  Y  G  Q  V  L  Y  T  D  K  T  Y    206
 781 GCCATGGGACATCTAATTCAGAGGAAGAAGGTCCATGTCTTTGGGGATGAATTGAGTCTG  840
 207 A  M  G  H  L  I  Q  R  K  K  V  H  V  F  G  D  E  L  S  L    226
 841 GTGACTTTGTTTCGATGTATTCAAAATATGCCTGAAACACTACCCAATAATTCCTGCTAT  900
 227 V  T  L  F  R  C  I  Q  N  M  P  E  T  L  P  N  N  S  C  Y    246
 901 TCAGCTGGCATTGCAAAACTGGAAGAAGGAGATGAACTCCAACTTGCAATACCAAGAGAA  960
 247 S  A  G  I  A  K  L  E  E  G  D  E  L  Q  L  A  I  P  R  E    266
 961 AATGCACAAATATCACTGGATGGAGATGTCACATTTTTTGGTGCATTGAAACTGCTGTGA 1020
 267 N  A  Q  I  S  L  D  G  D  V  T  F  F  G  A  L  K  L  L  *    286
1021 CCTACTTACACCATGTCTGTAGCTATTTTCCTCCCTTTCTCTGTACCTCTAAGAAGAAAG 1080
1081 AATCTAACTGAA  1092
```

FIG. 11A

Nucleotide and amino acid sequence of mTNRL1-α

```
   1  CAGGAGAGCGCTCCTGGGGGAACCCAGCCCTGCCATGCTCTGAGGGCAGTCTCCCAGGAC   60
  61  ACAGATGACAGGAAATGACCCACCCCTGTGGTCACTTACTCCAAAGGCCTAGACCTTCAA  120
 121  AGTGCTCCTCGTGGAATGGATGAGTCTGCAAAGACCCTGCCACCACCGTGCCTCTGTTTT  180
  -4                      M  D  E  S  A  K  T  L  P  P  P  C  L  C  F   15
 181  TGCTCCGAGAAAGGAGAAGATATGAAAGTGGGATATGATCCCATCACTCCGCAGAAAGAG  240
  16  C  S  E  K  G  E  D  M  K  V  G  Y  D  P  I  T  P  Q  K  E   35
 241  GAGGGTGCCTGGTTTGGGATCTGCAGGGATGGAAGGCTGCTGGCTGCTACCCTCCTGCTG  300
  36  E  G  A  W  F  G  I  C  R  D  G  R  L  L  A  A  T  L  L  L   55
 301  GCCCTGTTGTCCAGCAGTTTCACAGCGATGTCCTTGTACCAGTTGGCTGCCTTGCAAGCA  360
  56  A  L  L  S  S  S  F  T  A  M  S  L  Y  Q  L  A  A  L  Q  A   75
 361  GACCTGATGAACCTGCGCATGGAGCTGCAGAGCTACCGAGGTTCAGCAACACCAGCCGCC  420
  76  D  L  M  N  L  R  M  E  L  Q  S  Y  R  G  S  A  T  P  A  A   95
 421  GCGGGTGCTCCAGAGTTGACCGCTGGAGTCAAACTCCTGACACCGGCAGCTCCTCGACCC  480
  96  A  G  A  P  E  L  T  A  G  V  K  L  L  T  P  A  A  P  R  P  115
 481  CACAACTCCAGCCGCGGCCACAGGAACAGACGCGCTTTCCAGGGACCAGAGGAAACAGAA  540
 116  H  N  S  S  R  G  H  R  N  R  R  A  F  Q  G  P  E  E  T  E  135
 541  CAAGATGTAGACCTCTCAGCTCCTCCTGCACCATGCCTGCCTGGATGCCGCCATTCTCAA  600
 136  Q  D  V  D  L  S  A  P  P  A  P  C  L  P  G  C  R  H  S  Q  155
 601  CATGATGATAATGGAATGAACCTCAGAAACAGAACTTACACATTTGTTCCATGGCTTCTC  660
 156  H  D  D  N  G  M  N  L  R  N  R  T  Y  T  F  V  P  W  L  L  175
 661  AGCTTTAAAAGAGGAAATGCCTTGGAGGAGAAAGAGAACAAAATAGTGGTGAGGCAAACA  720
 176  S  F  K  R  G  N  A  L  E  E  K  E  N  K  I  V  V  R  Q  T  195
 721  GGCTATTTCTTCATCTACAGCCAGGTTCTATACACGGACCCCATCTTTGCTATGGGTCAT  780
 196  G  Y  F  F  I  Y  S  Q  V  L  Y  T  D  P  I  F  A  M  G  H  215
 781  GTCATCCAGAGGAAGAAAGTACACGTCTTTGGGGACGAGCTGAGCCTGGTGACCCTGTTC  840
 216  V  I  Q  R  K  K  V  H  V  F  G  D  E  L  S  L  V  T  L  F  235
 841  CGATGTATTCAGAATATGCCCAAAACACTGCCCAACAATTCCTGCTACTCGGCTGGCATC  900
 236  R  C  I  Q  N  M  P  K  T  L  P  N  N  S  C  Y  S  A  G  I  255
 901  GCGAGGCTGGAAGAAGGAGATGAGATTCAGCTTGCAATTCCTCGGGAGAATGCACAGATT  960
 256  A  R  L  E  E  G  D  E  I  Q  L  A  I  P  R  E  N  A  Q  I  275
 961  TCACGCAACGGAGACGACACCTTCTTTGGTGCCCTAAAACTGCTGTAACTCACTTGCTGG 1020
 276  S  R  N  G  D  D  T  F  F  G  A  L  K  L  L  *              295
1021  AGTGCGTGATCCCCTTCCCTCGTCTTCTCTGTACCTCCGAGGGAGAAACAGACGACTGGA 1080
1081  AAAACTAAAAGATGGGGAAAGCCGTCAGCGAAAGTTTTCTCGTGACCCGTTGAATCTGAT 1140
1141  CCAAACCAGGAAATATAACAGACAGCCACAACCGAAGTGTGCCATGTGAGTTATGAGAAA 1200
1201  CGGAGCCCGCGCTCAGAAAGACCGGATGAGGAAGACCGTTTTCTCCAGTCCTTTGCCAAC 1260
1261  ACGCACCGCAACCTTGCTTTTTGCCTTGGGTGACACATGTTCAGAATGCAGGGAGATTTC 1320
1321  CTTGTTTTGCGATTTGCCATGAGAAGAGGGCCCACAACTGCAGGTCACTGAAGCATTCAC 1380
1381  GCTAAGTCTCAGGATTTACTCTCCCATCACATGCTAAGTACACACACGCTCTTTTCCAGG 1440
1441  TAATACTATGGGATACTATGGAAAGGTTGTTTGTTTTTAAATCTAGAAGTCTTGAACTGG 1500
1501  CAATAGACA  1509
```

FIG. 11B

```
   1  ACAACCTTCTTCCCTTCTGCACCACTGCCCGTACCCTTACCCGCCCCGCCACCTCCTTGC  60
  61  TACCCCACTCTTGAAACCACAGCTGTTGGCAGGGTCCCCAGCTCATGCCAGCCTCATCTC  120
 -13                                                M  P  A  S  S  P   6
 121  CTTTCTTGCTAGCCCCCAAAGGGCCTCCAGGCAACATGGGGGGCCCAGTCAGAGAGCCGG  180
   7    F  L  L  A  P  K  G  P  P  G  N  M  G  G  P  V  R  E  P  A   26
 181  CACTCTCAGTTGCCCTCTGGTTGAGTTGGGGGCAGCTCTGGGGGCCGTGGCTTGTGCCA  240
  27    L  S  V  A  L  W  L  S  W  G  A  A  L  G  A  V  A  C  A  M   46
 241  TGGCTCTGCTGACCCAACAAACAGAGCTGCAGAGCCTCAGGAGAGAGGTGAGCCGGCTGC  300
  47    A  L  L  T  Q  Q  T  E  L  Q  S  L  R  R  E  V  S  R  L  Q   66
 301  AGGGGACAGGAGGCCCCTCCCAGAATGGGGAAGGGTATCCCTGGCAGAGTCTCCCGGAGC  360
  67    G  T  G  G  P  S  Q  N  G  E  G  Y  P  W  Q  S  L  P  E  Q   86
 361  AGAGTTCCGATGCCCTGGAAGCCTGGGAGAGTGGGGAGAGATCCCGGAAAAGGAGAGCAG  420
  87    S  S  D  A  L  E  A  W  E  S  G  E  R  S  R  K  R  R  A  V  106
 421  TGCTCACCCAAAAACAGAAGAAGCAGCACTCTGTCCTGCACCTGGTTCCCATTAACGCCA  480
 107    L  T  Q  K  Q  K  K  Q  H  S  V  L  H  L  V  P  I  N  A  T  126
 481  CCTCCAAGGATGACTCCGATGTGACAGAGGTGATGTGGCAACCAGCTCTTAGGCGTGGGA  540
 127    S  K  D  D  S  D  V  T  E  V  M  W  Q  P  A  L  R  R  G  R  146
 541  GAGGCCTACAGGCCCAAGGATATGGTGTCCGAATCCAGGATGCTGGAGTTTATCTGCTGT  600
 147    G  L  Q  A  Q  G  Y  G  V  R  I  Q  D  A  G  V  Y  L  L  Y  166
 601  ATAGCCAGGTCCTGTTTCAAGACGTGACTTTCACCATGGGTCAGGTGGTGTCTCGAGAAG  660
 167    S  Q  V  L  F  Q  D  V  T  F  T  M  G  Q  V  V  S  R  E  G  186
 661  GCCAAGGAAGGCAGGAGACTCTATTCCGATGTATAAGAAGTATGCCCTCCCACCCGGACC  720
 187    Q  G  R  Q  E  T  L  F  R  C  I  R  S  M  P  S  H  P  D  R  206
 721  GGGCCTACAACAGCTGCTATAGCGCAGGTGTCTTCCATTTACACCAAGGGGATATTCTGA  780
 207    A  Y  N  S  C  Y  S  A  G  V  F  H  L  H  Q  G  D  I  L  S  226
 781  GTGTCATAATTCCCCGGGCAAGGGCGAAACTTAACCTCTCTCCACATGGAACCTTCCTGG  840
 227    V  I  I  P  R  A  R  A  K  L  N  L  S  P  H  G  T  F  L  G  246
 841  GGTTTGTGAAACTGTGATTGTGTTATAAAAAGTGGCTCCCAGCTTGGAAGACCAGGGTGG  900
 247    F  V  K  L  *
 901  GTACATACTGGAGACAGCCAAGAGCTGAGTATATAAAGGAGAGGGAATGTGCAGGAACAG  960
 961  AGGCGTCTTCCTGGGTTTGGCTCCCCGTTCCTCACTTTTCCCTTTTCATTCCCACCCCCT  1020
1021  AGACTTTGATTTTACGGATATCTTGCTTCTGTTCCCCATGGAGCTCCGAATTCTTGCGTG  1080
1081  TGTGTAGATGAGGGGCGGGGGACGGGCGCCAGGCATTGTCCAGACCTGGTCGGGGCCCAC  1140
1141  TGGAAGCATCCAGAACAGCACCACCATC  1168
```

FIG. 12A

```
  1  GTTGGCAGGGTCCCTAGCTCATGCCAGCCTCATCTCCAGGCCACATGGGGGGCTCAGTCA  60
 -5                          M  P  A  S  S  P  G  H  M  G  G  S  V  R  14
 61  GAGAGCCAGCCCTTTCGGTTGCTCTTTGGTTGAGTTGGGGGGCAGTTCTGGGGGCTGTGA  120
 15    E  P  A  L  S  V  A  L  W  L  S  W  G  A  V  L  G  A  V  T  34
121  CTTGTGCTGTCGCACTACTGATCCAACAGACAGAGCTGCAAAGCCTAAGGCGGGAGGTGA  180
 35    C  A  V  A  L  L  I  Q  Q  T  E  L  Q  S  L  R  R  E  V  S  54
181  GCCGGCTGCAGCGGAATGGAGGGCCTTCCCAGAAGCAGGGAGAGCGCCCATGGCAGAGCC  240
 55    R  L  Q  R  N  G  G  P  S  Q  K  Q  G  E  R  P  W  Q  S  L  74
241  TCTGGGAGCAGAGTCCTGATGTCCTGGAAGCCTGGAAGGATGGGGCGAAATCTCGGAGAA  300
 75    W  E  Q  S  P  D  V  L  E  A  W  K  D  G  A  K  S  R  R  R  94
301  GGAGAGCAGTACTCACCCAGAAGCACAAGAAGAAGCACTCAGTCCTGCATCTTGTTCCAG  360
 95    R  A  V  L  T  Q  K  H  K  K  K  H  S  V  L  H  L  V  P  V  114
361  TTAACATTACCTCCAAGGCAGACTCTGACGTGACAGAGGTGATGTGGCAACCAGTACTTA  420
115    N  I  T  S  K  A  D  S  D  V  T  E  V  M  W  Q  P  V  L  R  134
421  GGCGTGGGAGAGGCCTGGAGGCCCAGGGAGACATTGTACGAGTCTGGGACACTGGAATTT  480
135    R  G  R  G  L  E  A  Q  G  D  I  V  R  V  W  D  T  G  I  Y  154
481  ATCTGCTCTATAGTCAGGTCCTGTTTCATGATGTGACTTTCACAATGGGTCAGGTGGTAT  540
155    L  L  Y  S  Q  V  L  F  H  D  V  T  F  T  M  G  Q  V  V  S  174
541  CTCGGGAAGGACAAGGGAGAAGAGAAACTCTATTCCGATGTATCAGAAGTATGCCTTCTG  600
175    R  E  G  Q  G  R  R  E  T  L  F  R  C  I  R  S  M  P  S  D  194
601  ATCCTGACCGTGCCTACAATAGCTGCTACAGTGCAGGTGTCTTTCATTTACATCAAGGGG  660
195    P  D  R  A  Y  N  S  C  Y  S  A  G  V  F  H  L  H  Q  G  D  214
661  ATATTATCACTGTCAAAATTCCACGGGCAAACGCAAAACTTAGCCTTTCTCCGCATGGAA  720
215    I  I  T  V  K  I  P  R  A  N  A  K  L  S  L  S  P  H  G  T  234
721  CATTCCTGGGGTTTGTGAAACTATGATTGTTATAAAGGGGGTGGGGATTTCCCATTCCAA  780
235    F  L  G  F  V  K  L  *                                        254
781  AAACTGGCTAGACAAAGGACAANGAAACGGTCNANAACANCTCTCCATGGCTTTGCCTTG  840
841  ACTGTTGTTCCTCCCTTTGCCTTTCCCCGCTCCCNCTATCTGGGCTTTGACTCCCTGGGA  900
901  TATTAAAAAANTTNAATATTTTNGTGTTTATCTCCC                          936
```

FIG. 12B

```
1     GTCAGTTTGGGGAGCCGGGCATCGCTGTCCGCCCAGGAGCCTGCCCAGGAGGAGCTGGTG  60
1     V  S  L  G  S  R  A  S  L  S  A  Q  E  P  A  Q  E  E  L  V    20
61    GCAGAGGAGGACCAGGACCCGTCGGAACTGAATCCCCAGACAGAAGAAAGCCAGGATCCT  120
21    A  E  E  D  Q  D  P  S  E  L  N  P  Q  T  E  E  S  Q  D  P    40
121   GCGCCTTTCCTGAACCGACTAGTTCGGCCTCGCAGAAGTGCACCTAAAGGCCGGAAAACA  180
41    A  P  F  L  N  R  L  V  R  P  R  R  S  A  P  K  G  R  K  T    60
181   CGGGCTCgAaGAGCGATCGCAGCCCATTATGAAGTTCATCCACGACCTGGACAGGACGGA  240
61    R  A  R  R  A  I  A  A  H  Y  E  V  H  P  R  P  G  Q  D  G    80
241   GCGCAGGCAGGTGTGGACGGGACAGTGAGTGGCTGGGAGGAAGCCAGAATCAACAGCTCC  300
81    A  Q  A  G  V  D  G  T  V  S  G  W  E  E  A  R  I  N  S  S    100
301   AGCCCTCTGCGCTACTACCGCCAGATCGGGGAGTTTATAGTCACCCGGGCTGGGCTCTAC  360
101   S  P  L  R  Y  Y  R  Q  I  G  E  F  I  V  T  R  A  G  L  Y    120
361   TACCTGTACTGTCAGGTGCACTTTGATGAGGGGAAGGCTGTCTACCTGAAGCTGGACTTG  420
121   Y  L  Y  C  Q  V  H  F  D  E  G  K  A  V  Y  L  K  L  D  L    140
421   CTGGTGGATGGTGTGCTGGCCCTGCGCTGCCTGGAGGAATTCTCAGCCACTGCGGCCAGT  480
141   L  V  D  G  V  L  A  L  R  C  L  E  E  F  S  A  T  A  A  S    160
481   TCCCTCGGGCCCCAGCTCCGCCTCTGCCAGGTGTCTGGGCTGTTGGCCcTGCGGcCAGGG  540
161   S  L  G  P  Q  L  R  L  C  Q  V  S  G  L  L  A  L  R  P  G    180
541   TCCTCCCTGCGGATCCGCACCCTCCCCTGGGCCCATCTCAAGGCTGCCCCCTTCCTCACC  600
181   S  S  L  R  I  R  T  L  P  W  A  H  L  K  A  A  P  F  L  T    200
601   TACTTCGGACTCTTCCAGGTTCACTGAGGGGCCCTGGTCTCCCCACAGTCGTCCCAGGCT  660
201   Y  F  G  L  F  Q  V  H  *                                     220
661   GCCGGCTCCCCTCGACAGCTCTCTGGGCACCCGGTCCCCTCTGCCCCACCCTCAGCCGCT  720
721   CTTTGCTCCAGACCTGCCCCTCCCTCTAGAGGCTGCCTGGGCCTGTTCACGTGTTTTCCA  780
781   TCCCACATAAATACAGTATTCCCACTCTTATCTTACAACTCCCCCACCGCCCACTAAGAG  840
841   GGGCTGGACTGGCGGCAGGAAGCCAAAGAGACTGGCCTAGGCCAGGAGTTCCCAAATGTG  900
901   AGGGGCGAGAAACAAGACAAGCTCCTCCCTTGAGAATTCCCTGTGGATTTTTAAAACAGA  960
961   TATTATTTTTATTATTATTGTGACAAAATGTTGATAAATGGATATTAAATAGAAAAAAAA  1020
1021  AAAAAAAAAA
```

FIG. 13A

```
1    CTGGTCGTGGTCAGCCTGGGGAGCTGGGCAACGCTGTCTGCCCAGGAGCCTTCTCAGGAG   60
1     L  V  V  V  S  L  G  S  W  A  T  L  S  A  Q  E  P  S  Q  E    20
61   GAGCTGACAGCAGAGGACCGCCGGGAGCCCCCTGAACTGAATCCCCAGACAGAGGAAAGC  120
21    E  L  T  A  E  D  R  R  E  P  P  E  L  N  P  Q  T  E  E  S    40
121  CAGGATGTGGTACCTTTCTTGGAACAACTAGTCCGGCCTCGAAGAAGTGCTCCTAAAGGC  180
41    Q  D  V  V  P  F  L  E  Q  L  V  R  P  R  R  S  A  P  K  G    60
181  CGGAAGGCGCGGCCTCGCCGAGCTATTGCAGCCCATTATGAGGTTCATCCTCGGCCAGGA  240
61    R  K  A  R  P  R  R  A  I  A  A  H  Y  E  V  H  P  R  P  G    80
241  CAGGATGGAGCACAAGCAGGTGTGGATGGGACAGTGAGTGGCTGGGAAGAGACCAAAATC  300
81    Q  D  G  A  Q  A  G  V  D  G  T  V  S  G  W  E  E  T  K  I   100
301  AACAGCTCCAGCCCTCTGCGCTACGACCGCCAGATTGGGGAATTTACAGTCATCAGGGCT  360
101   N  S  S  S  P  L  R  Y  D  R  Q  I  G  E  F  T  V  I  R  A   120
361  GGGCTCTACTACCTGTACTGTCAGGTGCACTTTGATGAGGGAAAGGCTGTCTACCTGAAG  420
121   G  L  Y  Y  L  Y  C  Q  V  H  F  D  E  G  K  A  V  Y  L  K   140
421  CTGGACTTGCTGGTGAACGGTGTGCTGGCCCTGCGCTGCCTGGAAGAATTCTCAGCCACA  480
141   L  D  L  L  V  N  G  V  L  A  L  R  C  L  E  E  F  S  A  T   160
481  GCAGCAAGCTCTCCTGGGCCCCAGCTCCGTTTGTGCCAGGTGTCTGGGCTGTTGgCGCTG  540
161   A  A  S  S  P  G  P  Q  L  R  L  C  Q  V  S  G  L  L  A  L   180
541  CGGcCAGGGTCTTCCCTTCGGATCCGCACCCTCCCCTGGGCTCATCTTAAGGCTGCCCCC  600
181   R  P  G  S  S  L  R  I  R  T  L  P  W  A  H  L  K  A  A  P   200
601  TTCCTAACCTACTTTGGACTCTTTCAAGTTCACTGAGGGGCCTTGCTCTCCCAGATTCCT  660
201   F  L  T  Y  F  G  L  F  Q  V  H  *                           220
661  TAAACTTTCCCTGGCTCCAGGAGCATCACCACACCTCCCTA  701
```

FIG. 13B

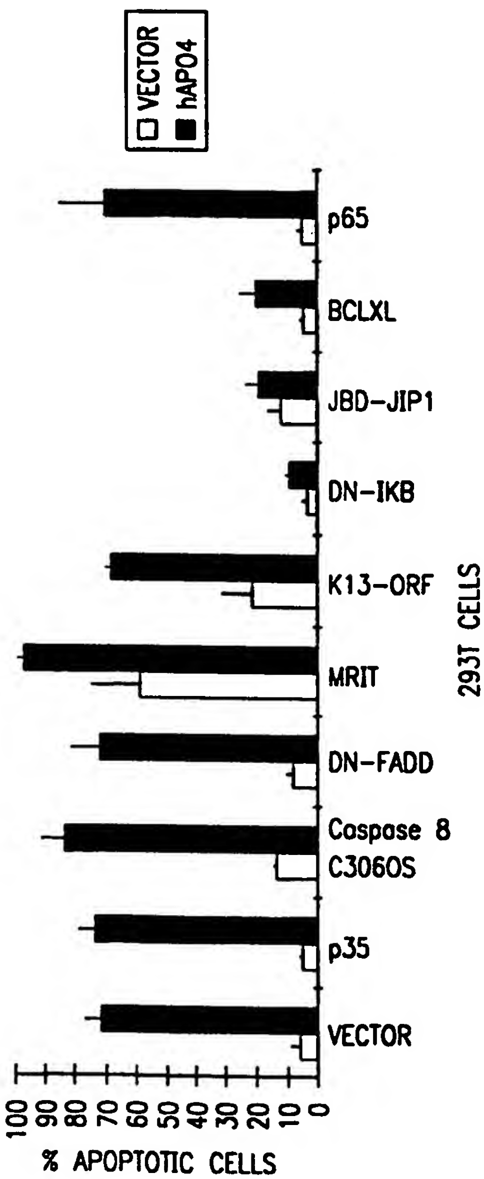

FIG. 14A

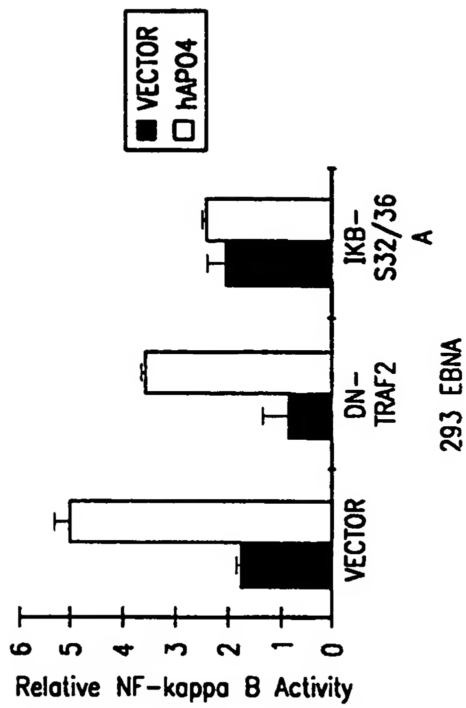

FIG. 14C

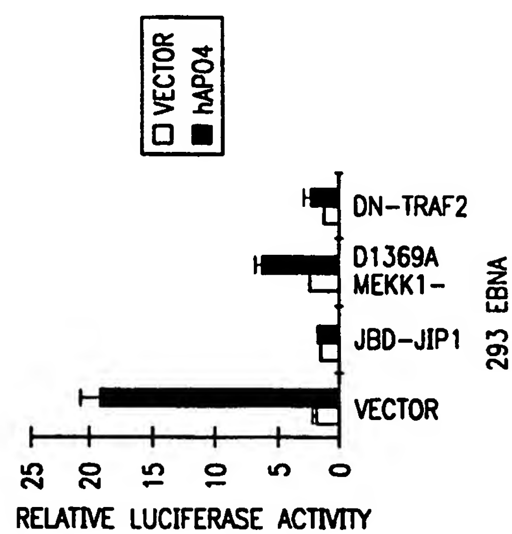

FIG. 14B